

STUDY : Tj 289/03-2070

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**ORAL TOXICITY TEST AFTER 28-DAY REPEATED
ADMINISTRATION IN THE RAT OF A FOOD SUPPLEMENT
ING 911**

RAPPORT D'ESSAI

Blanquefort, November 10, 2003

PhD/CG

71 page document

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1. AIM AND PRINCIPLE OF THE TEST

The aim of the test was to assess qualitatively and quantitatively the toxic phenomena and the rate of onset after repeated administration, by oral route in the Rat, of the ING 911 product, (Caseine α S1 hydrolysate) used as a food supplement.

The test preparation was orally administered daily at graduated doses to several animals' groups (treated groups), one dose being used per group for a period of 28 consecutive days.

Concurrently, one animals' group (control group) received, the same volume of vehicle under the same conditions for 28 consecutive days.

During the administration period, the animals were closely observed each day for signs of toxicity. Biochemical and haematological examinations were performed at the end of treatment, on all the animals.

Then animals were sacrificed to be necropsied and subjected to appropriate histopathological examinations.

Rat is the rodent species commonly used and recommended by official authorities for the assessment of chemical and medicinal substances safety by this type of method.

The treatment repeated for 28 days by oral route is one of the major methods retained by the authorities for the assessment of the exposure risk and the calculation of the safety margin peculiar to a given pharmaceutical substance.

This test was performed according to the principles of the Good Laboratory Practices following the study plan ref. PSp. 03-0444/1 of July 4, 2003 reported in Appendix 10.

The methodology followed the OECD guideline No 407 of July 27, 1995 and the Appendix IV.D part B7 of the European Directive 96/54/EEC of July 30, 1996 published in the Official Journal of the European Communities of September 30, 1996 (L248).

The test began on July 07, 2003 and was achieved on September 29, 2003 with the histological examination.

2. TEST FACILITY AND PARTNERS

2.1. Test facility and technical staffs

EVIC France – Division Evic-Tox
48 rue Jean Duvert
33290 Blanquefort
05 56 95 59 95

Study Director : Philippe DUFOUR
Responsible technician : Martine MIERMON

GREF/INSERM E9917
Université Victor Segalen – Bordeaux 2
146 rue Léo Saignat
33076 Bordeaux Cedex
05 57 57 17 71

Anatomopathologist : Rosa URBANIAK

2.2. Partners

Biochemical analyses (Na/K)
Laboratoire d'Analyses de Biologie Médicale Ruffié et Associés Doctor Biologist : Ch. DUBOIS
17 allées de Tourny
33080 Bordeaux Cedex
05 56 79 45 00

Haematological and biochemical analyses

EVIC France – Division Evic-Bio
48 rue Jean Duvert
33290 Blanquefort
05 56 95 59 95

Responsible : M.A. ALONSO

2.3. Ethics and approval of the Test Facility

The test was entirely performed according to the animal ethical rules mentioned in the **European Directive 86/609/EEC** of November 24, 1986 and was submitted to the previous agreement of the animal Ethics Committee internal to the Test Facility.

It was conducted according to the internal rules of the Quality System of EVIC france company, which was declared in conformity with the GLP by the **AFSSAPS** (decree of March 14, 2000 published in the Official Journal of the French Republic of March 23, 2000) and the **GIPC** (decree No 98-1312 of December 31, 1998 published in the Official Journal of the French Republic of January 1st, 1999) and with the NF EN ISO/CEI 17025 standard by the **COFRAC** (accreditation No 1-0042).

3. QUALITY ASSURANCE

All the data collected during the test were recorded by the technician responsible for the test, on the documents reserved for that effect.

Each page of these documents was initialled and dated by the technician responsible for the test. Any missing data was justified and the corrections were initialled and dated.

The Quality Assurance Unit ensured by periodic audits that the study plan and working procedures relevant to this type of test were strictly applied.

Any modification to the study plan was submitted to an amendment signed by the Sponsor and the Study Director.

The Quality Assurance Unit performed regular audits about the Test Facility in compliance with the corresponding procedure.

The experimental data and the test report were audited in accordance with the procedure implemented in the Test Facility.

At the end of the test, the work documents were filed with the test report and histological samples for 10 years in the filling room of the Test Facility.

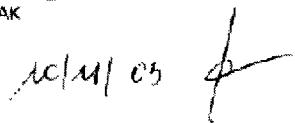
At the end of this period, the Test Facility defines with the Sponsor the carrying out of the filing, the restitution or the destruction of the data and histological samples.

4. STUDY RESPONSIBLE PERSONNEL'S STATEMENT**Test Facility Management**

I the undersigned, **Philippe DUFOUR**, declare that the overall of the study was carried out under my responsibility and in accordance with the principles of Good Laboratory Practices according to the rules appropriate to this study.



Anatomopathologist
Rosa URBANIAK

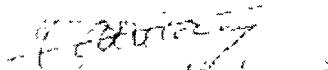

Quality Assurance

I the undersigned, **Michèle DARRICAU**, declare that :

- according to the procedure of the Test Facility, the study was audited during various phases of its performing and that the audit reports were transmitted to the Management and to the Study Director on the dates listed in the following table :

Audit date	Phase audited	Report date
09.07.03	Preparation of the product before treatment Male and female treatment (D1-D2)	15.07.03
05.08.03	Haematology - blood sampling from the retro-orbital sinus	11.08.03
05.08.03	Necropsy - males	11.08.03
06.08.03	Haematology - platelet count	11.08.03
11.09.03	Biochemical assays	15.09.03
22.09.03	Histological preparations (blocks)	29.09.03

- the draft of the final report was audited on October 15, 2003, and the present report examined on November 10, 2003,
- the results reported accurately and completely reflect the raw data of the study



5. SUMMARY

Aim of the test

The objective of this study was to assess the toxicity of a test preparation following daily oral (gavage) administration to the Sprague Dawley Rat for 28 consecutive days.

The test was conducted in compliance with the principles of the Good Laboratory Practices (Directive 87/18/EEC).

Test preparation

ING 911 - batch 06/03

Reactive system

40 (20 males and 20 females) Sprague Dawley Rat : ICO.OFA.SD (IOPS caw).

Methods

Animals were allocated into 4 groups of 5 males and 5 females (groups 1 to 4).

The test preparation suspended in distilled water was administered orally (by gavage) at the doses of 1000 mg/kg/day (group 4), 200 mg/kg/day (group 3) and 40 mg/kg/day (group 2) for 28 consecutive days, 7 days a week.

Control animals (group 1) received 10 ml/kg/day of the vehicle (distilled water) under the same conditions.

The animals were regularly observed throughout the test period (mortality, clinical signs, body weight, food consumption).

Haematological and biochemical investigations were performed at termination.

At the end of the test, all animals were killed and necropsied, a range of organs was weighed and selected tissues were examined microscopically.

Results

Under the experimental conditions adopted, the results were :

Mortality

No mortality was observed during the treatment period.

Clinical observations

Throughout the test period, no clinical signs were observed in the groups receiving the test preparation comparing to the control group.

Body weight

Body weight growth of the animals receiving ING 911 was not altered ; it was not significantly different from that of control animals.

Food consumption

Food consumption of the treated animals was similar to that of control animals.

Hematology

Slight significant differences from controls were noted in treated animals (decrease in hematocrit and leucocytes, increase in prothrombine time). They were considered to be of no toxicological importance.

Biochemistry

Compared with controls, a small, but statistically significant increase in serum ASAT and ALAT was observed in male receiving 200 and 1000 mg/kg/day ING 911. This change was not considered to be biologically significant.

Gross necropsy

The treatment did not induce any significant macroscopic lesions.

Organ weight

The administration of ING 911 did not significantly affect the absolute and relative organ weights in male and female rats.

Histopathology

No target organ was evidenced.

Minor hepatic lesions were found identically in animals receiving 1000 mg/kg/day of ING 911 and in control animals.

Conclusion

Under the experimental conditions adopted, the preparation **ING 911** daily administered by oral route, to male and female Sprague Dawley rat for 28 consecutive days at the doses of 40 mg/kg/day, 200 mg/kg/day and 1000 mg/kg/day induced no significant toxic effect.

In this test, the dose level of ING 911 inducing no observable toxic effects can be considered as higher than 1000 mg/kg/day.

Ph. DUFOUR

Eurotox Registered Parmacologist-Toxicologist

6. TEST PRODUCTS

6.1. Test preparation

Name, reference : ING 911

Date of receipt : June 25, 2003

Quantity received, packaging : 500 g, aluminium thermosoldered sachet

Aspect : white powder

Reference in the Test Facility : 03-2070

Storage : the test product was stored in its original packaging, at ambient temperature and out of the light.

Sampling : a sample of the test product was filed in the samples library of the Test Facility as a reference where it will be kept until its expiry date or for a maximum period of 10 years.

6.2. Vehicle (control)

Distilled water for injection (10 ml ampoule) - Cooper batch 0007 (77020 Melun, France).

7. REACTIVE SYSTEM

Species : Sprague Dawley albino Rats : ICO.OFA.SD (IOPS caw)

Origin : Charles River Laboratories (69592 L'Arbresle Cedex, France)

Age : between 5 and 6 weeks (when put in acclimatization)

Number and sex : 40 allocated into four groups of 10 animals (5 males and 5 nulliparous and non-pregnant females)

Acclimatization : 11 days prior to the start of the test.

Allocation of the animals by group :

- *Weighing* : Prior to the test (D-1), the animals grouped according to their sex, were weighed. They were assigned to the treated group and control group by randomisation, according to the Moses and Oakford's random table. The homogeneity of the groups was confirmed by analysis of variance coefficients after allocation, on the basis of body weight. For each group (males or females), the mean weight was calculated and the acceptable limits were deduced, the extreme individual weights of the animals must not deviate from the mean group weight by more than $\pm 20\%$.
- *Identification* : the animals were identified individually per cage, by marking with picric acid. The location of the marking, different for each animal, corresponded to a number. A caudal marking represented by a coloured circle by means of a marker pen enabled to identify the group.

Housing : the animals were housed at the rate of 5 per cage, in 31 cm x 46 cm x 19 cm polypropylene cages with stainless steel lid.

The bedding renewed regularly, was composed of wood shavings delivered dust-free and sterilized to γ radiations. It was supplied by SICSA (94142 Alfortville, France).

The cages were placed in limited-access premises, of 5 m x 4.5 m x 3 m, maintained in slight overpressure (a minimum of 10 mm of water), under air-conditioned temperature ($t = 22 \pm 2^\circ\text{C}$) and controlled relative humidity (HR = 50 $\pm 20\%$) except during washing cycles and whose renewal in fresh filtered air (an absolute filter) was performed at the rate of $\text{phi} + 10\%$.

The artificial lighting ensured a sequence of 12 hours light, 12 hours dark.

Feeding : the complete diet was supplied under pelleted from (A04-10) delivered sterilised to γ radiations by UAR (Villemoisson, 91360 Epinay sur Orge, France). A food control sheet is supplied in Appendix 9.

Drinking : the acidified tap water ($\text{pH} = 2.5$) was distributed in polypropylene bottles with stainless steel teat. A sample of water was taken after each technical intervention from the pipes and every 6 months at least and sent for chemicophysical and bacteriological analysis to specialised control laboratory.

8. TEST PROCEDURE

8.1. Test preparation

The test preparation was suspended in water for injection and manually homogenised.

Each preparation performed **extemporaneously** each day in sufficient quantity for the test necessities was maintained under magnetic stirring during treatments.

The test preparation was transferred to the test room according to the modalities defined in the procedure of the Test Facility.

8.2. Dose levels - Number of groups

The dose levels were selected according to the results (no toxicity) obtained from a range finding test performed in the Test Facility on 6 animals (3 males and 3 females) receiving by gavage, 1000, 500 and 250 mg/kg/day dosage for 7 consecutive days and to the acute toxicity of the test preparation under a volume of 10 ml/kg.

Selected doses :

- high dose = **1000 mg/kg/day**
- medium dose = **200 mg/kg/day**
- low dose = **40 mg/kg/day**

The highest dosage (1000 mg/kg/day) corresponded to the minimum dose level recommended by the OECD Guideline No 407 for a limit test. It was the maximum dosage dispensable in good condition under the volume of 10 ml/kg (the volume administered should not exceed 1 ml/100 g body weight).

The low dose (40 mg/kg/day) corresponding to about 15 fold the daily dose level administered to humans would produce no observable adverse effects. The medium dosage (200 mg/kg/day) corresponded to the geometrical average from the 2 previous ones (increasing factor of 5).

Concurrently to the treated animals, the control animals handled exactly in the same way as the animals from treated groups, received a volume of vehicle (water for injection) equal to the volumes used in the treated groups (**10 ml/kg/day**).

The experimental groups were distributed as follows :

Groups	Treatment	ING 911 Dose levels(mg/kg/day)	Animals number/group	Identification of the animals	
				Males	Females
1	Water for injection (10 ml/kg)	0	10	9568 to 9572	9573 to 9577
2	ING 911 suspended in water for injection (4 mg/ml)	40	10	9578 to 9582	9583 to 9587
3	ING 911 suspended in water for injection (20 mg/ml)	200	10	9588 to 9592	9593 to 9597
4	ING 911 suspended in water for injection (100 mg/ml)	1000	10	9598 to 9602	9603 to 9607

8.3. Administration of the test preparation

The test preparation was administered to the animals daily, around the same hour (between 9:00 and 12:00 a.m.), seven days a week, over a period of 28 consecutive days.

The volume per kg of body weight being defined in a constant way for all dose levels (**10 ml/kg**) the volumes of the test suspension and vehicle were adjusted for each rat, weekly (on D1, D8, D15 and D22) on the criterion of the last weighing.

Each suspension was orally administered at one time, at a single dose, to each animal, by gavage using a 2.5 ml or 5 ml syringe fitted with a suitable sized cannula (76 mm x 15/10^e mm).

8.4. Treatment timetable

Animals were divided into 2 series :

- 1st serie : the 20 male rats were treated from July 08, 2003 (D1) to August 04, 2003 (D28).
- 2nd serie : the 20 female rats were treated from July 09, 2003 (D1) to August 05, 2003 (D28).

8.5. Control of the concentrations

On D1, D15 and D28 before gavage, after preparation of the test suspensions, one sample of each administered preparation was taken (3 different concentrations).

These samples were collected in labelled and dated 1.5 ml one use plastic microtubes and sent to the Sponsor Analytical Laboratory where analyses were carried out according to the appropriate methods. These samples were performed to check the concentration in active substance of the administered suspension.

8.6. Clinical examinations

8.6.1. Mortality

All animals were observed for morbidity and mortality, twice daily (morning and evening), except the public holidays (once only, in the morning).

8.6.2. Clinical observations

All the animals were observed during the treatment period (28 days).

A general clinical examination was performed once a day (at the same time of the day) before treatment then during the hour following gavage.

A detailed clinical examination was made in all animals :

- before the 1st treatment (D1/T0)
- and once a week during the test (D1, D8, D15, D22 and D28) within the hour following gavage.

The examinations were made outside the home cage in a standard area and always at the same time.

The different parameters observed included changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (lacrimation, piloerection, pupil size, unusual respiratory pattern...), changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes or bizarre behaviour. The sensory reactivity to stimuli of different types (auditory, visual and proprioceptive stimuli), grip strength and motor activity were assessed according to appropriate operative procedures.

8.6.3. Body weight

The animals were regularly weighed : during the acclimatization period (D-1) and on D1, D8, D15, D22 and D28, just before administrating the test preparation i.e. once a week throughout the test.

The day of the necropsy (D29), the animals were weighed fasting.

8.6.4. Food consumption

The quantity of food consumed was assessed per cage over a period of 48 hours, weekly by difference of weighing between the quantity of food supplied and the quantity of food remaining in bowls.

The results were expressed in g of food consumed/24 hours/100 g of body weight.

8.7. Laboratory examinations

8.7.1. Hematology

At the end of the treatment period (D28), all animals were fasted (hydrated diet) in the evening. On D29, animals were anaesthetised (Cloracetam®, 1 ml/kg i.m.) and blood samples were withdrawn from the retro-orbital sinus.

The blood was collected from all the animals to perform the following haematological examinations, according to the internal procedures of the Test Facility (methods listed in Appendix 3) :

- on EDTA : hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, erythrocyte indices (MCV, MCH, MCHC).
- on citrate : blood clotting time (Quick time).

8.7.2. Clinical biochemistry

After collections of blood for haematological examinations, all animals were anesthetized with Pentobarbital® (1,16 ml/kg, i.p.) and blood samples were taken from the abdominal aorta in each animal just prior to be killed.

The blood was collected into dry tubes, in order to perform the following biochemical assays, according to the internal procedures of the Test Facility (methods listed in Appendix 4) : glucose, creatinine, urea nitrogen, total protein, albumin, total cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatases (AP), calcium, sodium, potassium.

8.8. Anatomopathology

8.8.1. Gross necropsy

After blood samples collections for the clinical biochemistry, animals were sacrificed by bleeding at the abdominal aorta.

All the animals used in the test were subjected to a full detailed gross necropsy which included careful examination of the external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

The liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart of all the animals were trimmed of any adherent tissue and their wet weight taken as soon as possible after dissection. The paired organs were weighed separately.

The organ weight was expressed in absolute value and in relative value in comparison with brain weight

In all the rats, the following tissues or organs were sampled and preserved in an appropriate fixation medium (Bouin's fluid) : **all gross lesions, brain** (cerebrum, cerebellum and pons), **spinal cord, sciatic nerve** (close to the muscle), salivary glands, **stomach, small and large intestines** (including Peyer's patches), **liver, spleen, bone marrow** (at sternal level), **mesenteric lymph nodes, thymus, heart, aorta, trachea, lungs, thyroid /parathyroid, adrenals**, pancreas, pituitary, **kidneys, urinary bladder, gonads, epididymides, accessory sexual organs**, (uterus, prostate), vagina, mammary glands, bone (femur), and muscle (thigh).

8.8.2. Histopathology

All the macroscopic lesions, organs and tissues (in **bold** type) of all animals from the control group (group 1) and from the highest dosed group (group 4) were embedded in paraffin wax, cut at about 4 µm, stained with hemalun eosin and subjected to a full histopathological examination.

This examination may be performed on tissues and organs of animals from groups 2 and 3 in which changes were observed in the treated group 4.

8.9. Results

The results were expressed for each animal and were summarized under tabular form giving by group :

- the number of animals under test,
- the number of animals found dead during the test or killed for humanity reasons,
- the time of death or sacrifice,
- the number of animals showing signs of toxicity,
- the description, time of onset, duration and severity of these signs,
- the number of animals showing lesions,
- the kind of lesions and the number of animals with such lesions,
- body weight changes,
- food consumption,
- haematological, biochemical and pathological data.

Body weight changes were analysed separately for each sex by two-way analysis of variance for repeated measurements in time taking the "time" and "treatment factors" into consideration.

Haematology and clinical biochemistry data were analysed separately, parameter by parameter.

Once variance homogeneity between groups was confirmed (variation coefficient's analysis), the means were compared by analysis of variance.

If a statistically significant effect was found, the control group was compared to the treated groups using the Fisher's test.

Mean weights of tissues and organs removed on the necropsy day were analysed separately for each sex according to a process similar to the previous one.

Results of daily clinical findings, food consumption and macroscopic findings of organs at killing were discussed but not analysed statistically.

9. TEST TIMETABLE

Study plan approval by the Study Director	04.07.03
Animals arrival at the Test Facility.....	26.06.03
Treatment beginning (1 st serie)	08.07.03
Blood samples for haematological and biochemical examinations (1 st serie).....	05.08.03
Animals sacrifice (1 st serie)	05.08.03
Anatomopathology examination (end)	29.09.03

10. RESULTS

10.1. Control of the concentrations (Appendix 8)

In accordance with the Study plan, on D1, D15 and D28, after preparation of each suspension, one sample per dose was dispatched to the attention of M. J. TAUZIN, INGREDIA, 51-54 avenue F. Lobbedez – BP 946 – 62033 ARRAS Cedex – France under the responsibility of Evic France, at a time determined in agreement with the sponsor.

Results of analyses and quantity determination of the bioactive peptide by HPLC in ING 911 suspensions may be found in appendix 8.

Analyses of the study samples prepared on D1, D15 and 28 of the study indicated that the suspensions were prepared within \pm 7,2 % of the nominal concentrations. This was considered to be acceptable for the purpose of the study.

10.2. Clinical examinations

10.2.1. Mortality

No mortality was observed during the test period for the 4 animals' group.

10.2.2. Clinical observations

No signs of toxicity were noted during the test period. The general status and behaviour of the animals from the groups treated with ING 911 were always similar to those of animals from control group.

10.2.3. Body weight (Figure 1, Table 1, Appendix 1)

No significant differences were found between mean body weight of male and female rats receiving ING 911 and that of male and female rats from the control group. After the 28-day treatment, the mean body weight gain calculated for animals from the treated groups was similar to that of control animals.

10.2.4. Food consumption (Table 2, Appendix 2)

Food consumption of animals receiving ING 911 was quite similar to that of animals from the control group.

10.3. Laboratory examinations

10.3.1. Hematology (Table 3, Appendix 3)

Investigation of haematological parameters revealed a slight decrease in hematocrit (- 6,9 %) and Mean Corpuscular Volume (- 5 %) for males receiving 200 mg/kg/day ING 911 and in leucocyte count and lymphocytes (about 30 %) for males receiving 40 or 1000 mg/kg/day ING 911 in comparison with the controls. While these variations reached statistical significance ($p<0,05$), as the differences were small, and not dose-related, they were not considered to be toxicologically significant.

At 1000 mg/kg/day, in both sexes, the administration of ING 911 was associated with an increase in Prothrombine time (+ 7,8 % to + 17 % when compared with control mean values). This effect was not considered to be biologically significant, because all individual values noted in compound-treated animals (except for male 9598 from group 4) were near or within the range of values found in control animals.

A slight decrease in hematocrit was observed in females at 200 (- 6,9 %) and 1000 mg/kg/day (- 10.4 %) in comparison with the controls. These minimal changes, were not considered to be of toxicological importance because all individual values noted in compound treated animals (except for female 9605 from group 4) were near or within the range of values found in control animals and also, because mean values of hemoglobin and MCV in groups receiving 200 and 1000 mg/kg/day ING 911 were normal and not statistically different from those obtained for control animals.

In conclusion, these significant variations from controls which were, either slight or not dosage-related were considered to be no treatment-related or of no toxicological importance.

10.3.2. Clinical biochemistry (Table 4, Appendix 4)

After the 28-day treatment with ING 911, no significant difference was found between treated female animals and control female animals.

A small, but statistically significant increase in serum ASAT (maximum 86 %) and ALAT (maximum 46 %) was observed in males at 200 mg/kg/day and 1000 mg/kg/day when compared with control mean values.

However, this slight-to-mild elevation for liver enzymes, not dosage related, near or within the limits of control values, in the absence of any findings in liver weight and histopathology of this organ was considered to have no biological significance.

10.4. Anatomopathology

10.4.1. Gross necropsy (Table 5)

No macroscopically visible lesion, linked to treatment with ING 911 was observed. The few anomalies described (stomach ulcerations, discoloured liver) were found on a small number of animals of both sexes, from the treated and control groups.

10.4.2. Organ weight (Tables 6 and 7, Appendices 5 and 6)

Comparing to the animals from the control group 1, no significant change in absolute and relative organ weight was found in male or female animals treated with ING 911.

10.4.3. Histopathology (Table 8, Appendix 7)

No target organ was evidenced.

The examination of liver sections revealed a slight to moderate microvacuolation of hepatocytes in areas 1 and 2 lobules (RAPPAPORT lobules division) in 3/5 males and 5/5 females receiving 1000 mg/kg/day ING 911 and 1/5 male and 4/5 females of the control group. This minor anomaly found in the treated and control animals was considered to be of no toxicological significance because it is frequent for this animal species and partly due to fasting before sacrifice (glycogene draining).

Around certain centrilobular veins, some small diffuse granulomae were observed on 2/5 females receiving 1000 mg/kg/day ING 911 and 1/5 males and 2/5 females of the control group.

These lesions, visible in the treated and control animals, with the same frequency and severity were not considered to be treatment related.

No other histopathologic findings were noted in treated animals.

11. CONCLUSION

Under the experimental conditions adopted, the 28-day repeated administration of ING 911 by oral route in male and female Sprague Dawley Rat at the 40, 200 and 1000 mg/kg/day dosages induced no specific changes indicative of a treatment toxic effect.

Given these results, the 1000 mg/kg/day dose level of ING 911 may be considered, in this test, as the highest dose level where no adverse treatment related findings were observed.

Figure and Tables**Figure**

Figure 1 : Body weight – Mean values (g)

Tables

Table 1 : Body weight – Mean values (g)

Table 2 : Food consumption – Mean values (g/24 hours/100 g of body weight)

Table 3 : Hematology – Mean values

Table 4 : Biochemistry – Mean values

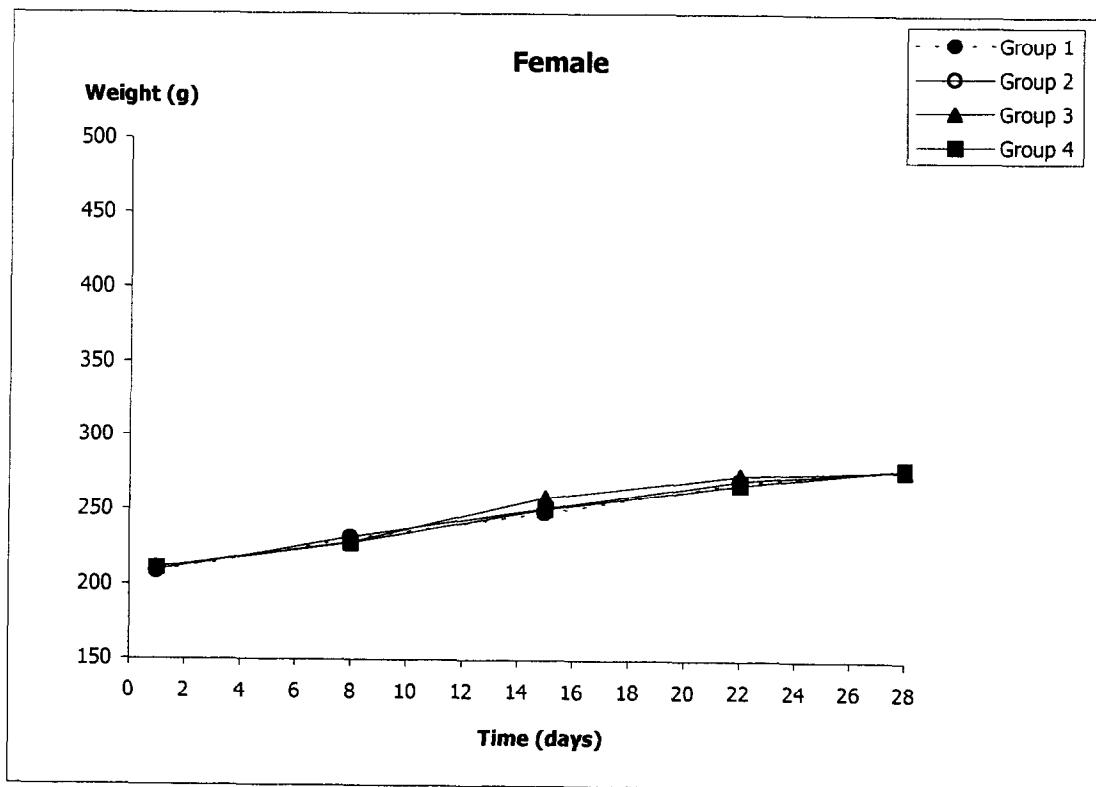
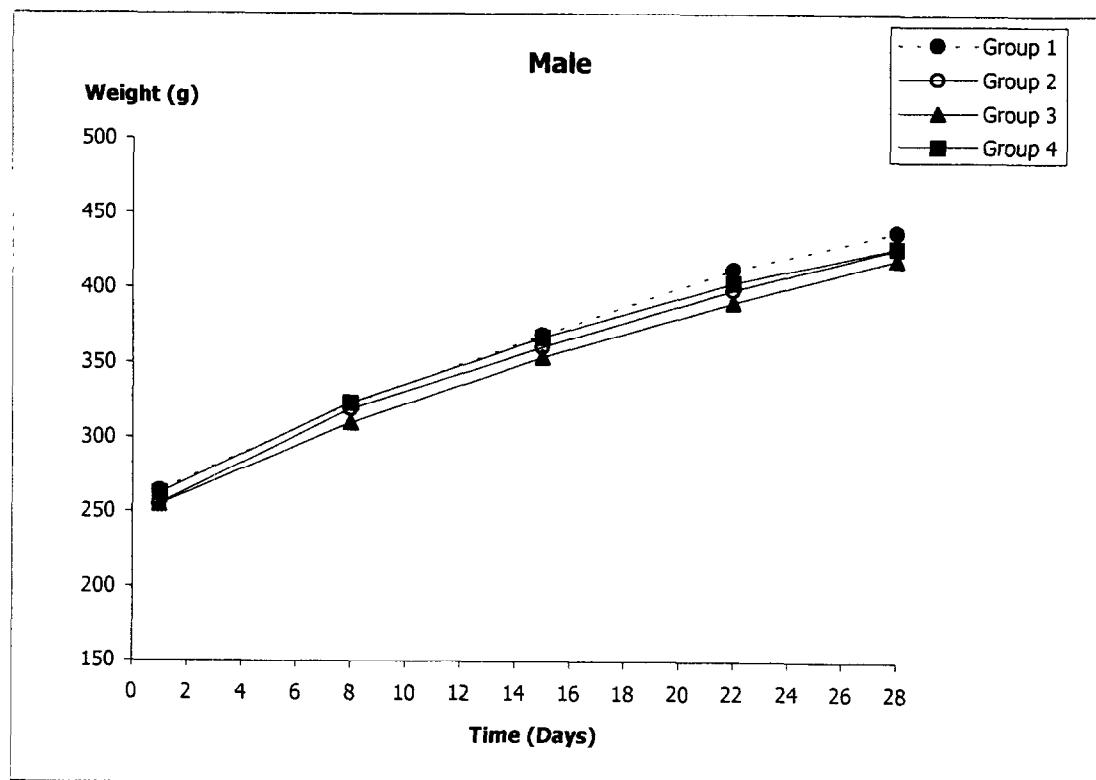
Table 5 : Necropsy findings – Individual observations

Table 6 : Absolute organ weight – Mean values (g)

Table 7 : Relative organ weight (brain weight ratio) - Mean values

Table 8 : Histopathological examination (% of animals showing abnormalities)

Figure 1
BODY WEIGHT - Mean values (g)



GROUP 1 : 10 ml/kg/jour, distilled water

GROUP 2 : 40 mg/kg/day, ING 911

GROUP 3 : 200 mg/kg/day, ING 911

GROUP 4 : 1000 ma/ka/dav ING 911

Table 1**BODY WEIGHT - Mean values (g)**

D1	D8	D15	D22	D28	D28-D1
----	----	-----	-----	-----	--------

Sex : Male

GROUP 1 10 ml/kg/day, distilled water						
n	5	5	5	5	5	5
Mean	264.1	322.8	368.9	412.8	438.4	174.2
SD	13.4	19.6	31.4	38.4	42.2	29.6
GROUP 2 40 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	255.6	319.4	361.0	399.0	427.2	171.6
SD	8.4	10.9	13.0	17.0	19.5	14.1
GROUP 3 200 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	254.8	310.2	354.3	390.6	419.7	164.9
SD	9.1	13.1	21.4	24.9	26.6	19.6
GROUP 4 1000 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	262.5	323.6	367.4	403.7	427.8	165.3
SD	9.9	16.6	22.2	25.3	28.8	20.8

Sex : Female

GROUP 1 10 ml/kg/day, distilled water						
n	5	5	5	5	5	5
Mean	208.6	230.9	248.6	269.7	278.2	69.7
SD	12.3	7.3	6.8	14.3	19.2	13.0
GROUP 2 40 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	209.7	232.2	251.8	270.8	278.6	68.9
SD	7.3	11.4	6.4	13.0	10.5	9.2
GROUP 3 200 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	211.6	228.7	259.0	274.2	277.9	66.3
SD	12.0	20.3	27.6	24.2	27.4	16.5
GROUP 4 1000 mg/kg/day, ING 911						
n	5	5	5	5	5	5
Mean	211.4	228.4	251.2	268.0	279.4	68.0
SD	15.2	11.9	9.0	7.9	11.5	12.8

* : p < 0.05, ** : p < 0.01, *** : p < 0.001 versus GROUP 1 (Fisher's test)

Table 2

**FOOD CONSUMPTION - Mean values
(g/24hours/100g of body weight)**

Sex : **Male**

	<i>Distilled water</i> 10 ml/kg/day GROUP 1	<i>ING 911</i>		
		40 mg/kg/day GROUP 2	200 mg/kg/day GROUP 3	1000 mg/kg/day GROUP 4
Week 1	10.4	10.3	10.0	10.0
Week 2	7.3	7.0	6.7	5.9
Week 3	6.9	7.9	7.6	7.1
Week 4	6.9	7.2	7.1	6.6

Sex : **Female**

	<i>Distilled water</i> 10 ml/kg/day GROUP 1	<i>ING 911</i>		
		40 mg/kg/day GROUP 2	200 mg/kg/day GROUP 3	1000 mg/kg/day GROUP 4
Week 1	8.5	9.0	8.4	8.9
Week 2	8.3	8.7	8.4	8.8
Week 3	8.7	9.4	7.8	7.7
Week 4	7.8	8.4	7.4	8.1

Table 3**HEMATOLOGY - Mean values**

Eryth T/l	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	Leuko G/I	Plat. G/I	Prothro. T (s) Ctrl 1	Prothro. T (s) Ctrl 2
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Sex : **Male**

GROUP 1 10 ml/kg/day, distilled water										
Mean	7.3	16.55	41.8	59	22.72	39.64	13.8	952	11.7	11.7
SD	0.3	1.36	1.1	3	1.47	3.43	2.8	111	0.6	0.6
GROUP 2 40 mg/kg/day, ING 911										
Mean	7.2	15.50	39.5	57	21.65	39.28	9.8	994	12.2	12.1
SD	0.4	0.85	2.9	2	0.59	1.36	1.2	66	0.3	0.2
GROUP 3 200 mg/kg/day, ING 911										
Mean	7.2	15.76	38.9	56	21.91	40.60	11.2	1022	12.5	12.3
SD	0.2	0.34	1.5	2	1.02	2.25	2.5	46	0.2	0.2
GROUP 4 1000 mg/kg/day, ING 911										
Mean	7.1	15.33	39.7	58	21.66	38.63	9.7	875	13.8	13.7
SD	0.3	0.74	2.0	3	0.53	1.68	2.1	48	2.9	2.7
			*				*			*

Sex : **Female**

GROUP 1 10 ml/kg/day, distilled water										
Mean	7.3	13.79	40.5	57	18.94	34.03	10.5	923	11.7	11.6
SD	0.5	1.23	2.5	2	1.04	2.26	1.8	130	0.5	0.6
GROUP 2 40 mg/kg/day, ING 911										
Mean	7.1	13.28	39.4	57	18.77	33.79	9.3	999	12.1	12.0
SD	0.3	1.34	2.2	1	1.84	3.91	1.4	73	0.5	0.4
GROUP 3 200 mg/kg/day, ING 911										
Mean	6.8	13.27	37.7	57	19.41	35.19	10.4	944	11.7	11.8
SD	0.3	0.47	1.2	2	0.51	0.87	2.4	106	0.4	0.3
GROUP 4 1000 mg/kg/day, ING 911										
Mean	7.0	13.03	36.3	54	18.52	35.96	8.8	879	12.4	12.5
SD	0.2	0.48	1.4	3	0.90	1.87	2.0	192	0.8	0.8
			**							*

*: p < 0.05, **: p < 0.01, ***: p < 0.001 versus GROUP 1 (Fisher's test)

Table 3 (continued)

HEMATOLOGY - Mean values

N %	E %	B %	L %	M %	Total Leuko G/l	N G/l	E G/l	B G/l	L G/l	M G/l
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Sex : Male

GROUP 1 10 ml/kg/day, distilled water											
Mean	11	0	0	89	1	13.8	1.49	0.03	0.00	12.15	0.09
SD	6	0	0	6	1	2.8	0.88	0.08	0.00	2.23	0.14
GROUP 2 40 mg/kg/day, ING 911											
Mean	17	1	0	82	0	9.8	1.63	0.08	0.00	8.05	0.00
SD	9	1	0	9	0	1.2	0.81	0.08	0.00	1.31	0.00
						*				**	
GROUP 3 200 mg/kg/day, ING 911											
Mean	12	0	0	88	0	11.2	1.25	0.05	0.00	9.85	0.06
SD	5	1	0	5	0	2.5	0.48	0.12	0.00	2.47	0.13
GROUP 4 1000 mg/kg/day, ING 911											
Mean	12	0	0	88	0	9.7	1.14	0.04	0.00	8.48	0.00
SD	3	1	0	3	0	2.1	0.34	0.06	0.00	1.84	0.00
						*				*	

Sex : Female

GROUP 1 10 ml/kg/day, distilled water											
Mean	7	1	0	91	1	10.5	0.74	0.12	0.00	9.59	0.08
SD	2	1	0	4	0	1.8	0.26	0.13	0.00	1.81	0.05
GROUP 2 40 mg/kg/day, ING 911											
Mean	10	1	0	89	0	9.3	0.92	0.13	0.00	8.27	0.00
SD	7	1	0	6	0	1.4	0.74	0.08	0.00	1.15	0.00
						*				***	
GROUP 3 200 mg/kg/day, ING 911											
Mean	12	1	0	87	0	10.4	1.24	0.07	0.00	9.11	0.00
SD	4	1	0	3	0	2.4	0.25	0.10	0.00	2.35	0.00
						*				***	
GROUP 4 1000 mg/kg/day, ING 911											
Mean	11	1	0	88	0	8.8	1.00	0.06	0.00	7.74	0.02
SD	2	1	0	2	0	2.0	0.24	0.07	0.00	1.82	0.04
						*				***	

*: p < 0.05, **: p < 0.01, ***: p < 0.001 versus GROUP 1 (Fisher's test)

Table 4
BIOCHEMISTRY -Mean values

	Gluc mmol/l	Crea μmol/l	Urea mmol/l	Chol mmol/l	Prot g/l	Alb g/l
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Sex : **Male**

GROUP 1	10 ml/kg/day, distilled water					
Mean	6.10	39.4	4.89	1.39	52.3	36.5
SD	0.57	1.2	0.84	0.37	1.0	1.1
GROUP 2	40 mg/kg/day, ING 911					
Mean	6.48	39.1	4.58	1.29	51.6	36.7
SD	0.67	2.7	0.54	0.12	2.4	1.0
GROUP 3	200 mg/kg/day, ING 911					
Mean	6.82	41.9	5.58	1.23	53.7	37.5
SD	1.14	3.5	1.44	0.22	2.5	1.6
GROUP 4	1000 mg/kg/day, ING 911					
Mean	6.85	40.7	5.25	1.26	54.0	37.2
SD	0.88	2.2	1.39	0.13	2.0	0.7

Sex : **Female**

GROUP 1	10 ml/kg/day, distilled water					
Mean	5.40	50.9	7.47	1.47	56.0	39.2
SD	0.68	10.6	1.69	0.32	2.7	2.5
GROUP 2	40 mg/kg/day, ING 911					
Mean	5.30	48.0	6.69	1.68	56.7	39.8
SD	0.44	3.0	1.25	0.41	4.3	2.5
GROUP 3	200 mg/kg/day, ING 911					
Mean	4.96	48.9	6.93	1.37	55.3	39.0
SD	0.92	3.0	1.07	0.22	2.5	1.6
GROUP 4	1000 mg/kg/day, ING 911					
Mean	5.13	48.4	7.45	1.36	55.7	38.2
SD	0.96	5.3	1.20	0.22	5.7	4.1

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs GROUP 1 (Fisher's test)

Table 4 (continued)**BIOCHEMISTRY -Mean values**

ASAT UI/l	ALAT UI/l	AP UI/l	Ca mmol/l	Na mmol/l	K mmol/l
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Sex : Male

GROUP 1		10 ml/kg/day, distilled water				
Mean	156	39	200	2.29	132	4.4
SD	30	8	38	0.07	2	0.2
GROUP 2		40 mg/kg/day, ING 911				
Mean	226	47	180	2.25	134	4.3
SD	45	9	34	0.03	3	0.1
GROUP 3		200 mg/kg/day, ING 911				
Mean	291	57	182	2.28	136	4.4
SD	71	13	55	0.08	2	0.3
	***	*				
GROUP 4		1000 mg/kg/day, ING 911				
Mean	284	56	225	2.24	137	4.3
SD	56	8	55	0.13	4	0.1
	**	*				

Sex : Female

GROUP 1		10 ml/kg/day, distilled water				
Mean	172	36	113	2.26	135	4.3
SD	67	12	25	0.13	4	0.4
GROUP 2		40 mg/kg/day, ING 911				
Mean	218	40	112	2.25	134	4.1
SD	62	7	32	0.07	3	0.4
GROUP 3		200 mg/kg/day, ING 911				
Mean	338	53	107	2.23	135	5.1
SD	274	42	40	0.07	3	1.5
GROUP 4		1000 mg/kg/day, ING 911				
Mean	278	47	99	2.19	135	4.4
SD	88	11	26	0.10	6	0.2

*: p < 0.05, **: p < 0.01, ***: p < 0.001 vs GROUP 1 (Fisher's test)

Table 5**NECROPSY FINDINGS - Individual observations**Sex : **Male**

Animals No	Anomalies noted
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GROUP 1 *10 ml/kg/day, distilled water*

9569 9572	Stomach : 2 ulcerations Stomach : 1 ulceration
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GROUP 2 *40 mg/kg/day, ING 911*

9582	Stomach : 1 ulceration
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GROUP 3 *200 mg/kg/day, ING 911*

9588 9589	Stomach : 1 ulceration Stomach : 1 ulceration
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GROUP 4 *1000 mg/kg/day, ING 911*

All of them	NTR
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NTR : nothing to report

Table 5 (continued)**NECROPSY FINDINGS - Individual observations**

Sex : **Female**

Animals No	Anomalies noted
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GROUP 1 *10 ml/kg/day, distilled water*

9573 9577	Urinary bladder : urine slightly pinkish Liver : discoloured
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GROUP 2 *40 mg/kg/day, ING 911*

All of them	NTR
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GROUP 3 *200 mg/kg/day, ING 911*

9596	Liver : discoloured
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GROUP 4 *1000 mg/kg/day, ING 911*

9606	Liver : discoloured
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NTR : nothing to report

Table 6
ABSOLUTE ORGAN WEIGHTS - Mean values (g)

Sex : Male

	Brain	Adrenal		Spleen	Liver	Kidney		Thymus	Heart	Testis		Epididymis	
		Left	Right			Left	Right			Left	Right	Left	Right
GROUP 1 10 ml/kg/day, distilled water													
Mean	1.985	0.028	0.027	0.732	11.581	1.469	1.468	0.511	1.375	1.484	1.455	0.515	0.518
SD	0.025	0.005	0.005	0.091	1.540	0.106	0.113	0.119	0.092	0.032	0.095	0.063	0.063
GROUP 2 40 mg/kg/day, ING 911													
Mean	2.059	0.026	0.027	0.671	11.099	1.415	1.406	0.487	1.335	1.547	1.550	0.478	0.495
SD	0.083	0.003	0.004	0.073	1.132	0.108	0.147	0.073	0.133	0.135	0.082	0.061	0.061
GROUP 3 200 mg/kg/day, ING 911													
Mean	2.034	0.028	0.028	0.642	11.315	1.454	1.421	0.476	1.319	1.597	1.627	0.531	0.555
SD	0.091	0.005	0.004	0.046	1.025	0.115	0.082	0.047	0.068	0.059	0.068	0.036	0.051
GROUP 4 1000 mg/kg/day, ING 911													
Mean	2.053	0.026	0.024	0.716	11.552	1.504	1.526	0.533	1.363	1.610	1.619	0.481	0.494
SD	0.091	0.003	0.003	0.084	1.234	0.097	0.128	0.078	0.097	0.143	0.144	0.022	0.038

Sex : Female

	Brain	Adrenal		Spleen	Liver	Kidney		Thymus	Heart
		Left	Right			Left	Right		
GROUP 1 10 ml/kg/day, distilled water									
Mean	1.934	0.035	0.031	0.499	7.150	0.882	0.864	0.434	0.944
SD	0.037	0.007	0.008	0.137	0.476	0.095	0.086	0.126	0.066
GROUP 2 40 mg/kg/day, ING 911									
Mean	1.938	0.035	0.036	0.537	7.118	0.889	0.896	0.527	0.936
SD	0.136	0.004	0.005	0.098	0.163	0.042	0.045	0.072	0.012
GROUP 3 200 mg/kg/day, ING 911									
Mean	2.005	0.037	0.035	0.529	7.401	0.931	0.934	0.453	0.907
SD	0.082	0.004	0.006	0.125	1.390	0.135	0.132	0.157	0.091
GROUP 4 1000 mg/kg/day, ING 911									
Mean	1.953	0.034	0.032	0.549	7.234	0.859	0.882	0.530	0.963
SD	0.049	0.002	0.006	0.115	0.312	0.018	0.020	0.096	0.105

* : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ vs GROUP 1 (Fisher's test)

Table 7

RELATIVE ORGAN WEIGHTS (brain weight ratio)
Mean values

Sex : Male

	Adrenal		Spleen	Liver	Kidney		Thymus	Heart	Testis		Epididymis	
	Left	Right			Left	Right			Left	Right	Left	Right
GROUP 1 10 ml/kg/day, distilled water												
Mean	1.43	1.36	36.92	583.97	74.06	73.97	25.74	69.25	74.73	73.25	25.93	26.12
SD	0.29	0.28	4.80	83.41	6.02	6.39	6.10	4.70	1.81	4.21	3.31	3.42
GROUP 2 40 mg/kg/day, ING 911												
Mean	1.26	1.30	32.60	539.01	68.70	68.30	23.65	65.01	75.04	75.29	23.17	23.97
SD	0.11	0.15	3.25	51.00	4.15	6.59	3.55	8.07	4.84	2.71	2.50	2.17
GROUP 3 200 mg/kg/day, ING 911												
Mean	1.38	1.36	31.59	558.34	71.55	69.88	23.39	64.95	78.63	80.08	26.19	27.37
SD	0.25	0.17	2.12	68.40	5.33	3.35	1.93	4.45	4.26	3.81	2.52	3.13
GROUP 4 1000 mg/kg/day, ING 911												
Mean	1.29	1.19	34.82	562.65	73.36	74.47	26.12	66.42	78.41	78.90	23.50	24.14
SD	0.15	0.17	3.49	55.75	5.62	7.70	4.96	4.63	6.09	6.95	1.93	2.75

Sex : Female

	Adrenal		Spleen	Liver	Kidney		Thymus	Heart
	Left	Right			Left	Right		
GROUP 1 10 ml/kg/day, distilled water								
Mean	1.81	1.62	25.76	369.59	45.57	44.67	22.40	48.84
SD	0.35	0.39	6.68	21.62	4.62	4.16	6.30	3.34
GROUP 2 40 mg/kg/day, ING 911								
Mean	1.83	1.85	27.69	368.51	46.07	46.38	27.15	48.46
SD	0.21	0.35	4.46	21.31	3.83	2.91	2.56	3.14
GROUP 3 200 mg/kg/day, ING 911								
Mean	1.85	1.76	26.26	367.76	46.32	46.49	22.48	45.15
SD	0.13	0.26	5.36	56.94	5.15	5.19	7.22	3.23
GROUP 4 1000 mg/kg/day, ING 911								
Mean	1.76	1.62	28.23	370.74	44.02	45.18	27.19	49.33
SD	0.09	0.33	6.58	22.67	1.88	1.38	5.20	5.42

*: p < 0.05, **: p < 0.01, ***: p < 0.001 vs GROUP 1 (Fisher's test)

Table 8

HISTOPATHOLOGICAL EXAMINATION (% of animals showing abnormalities)

Organ	Observations	Males		Females	
		5 Als Group 1	5 Als Group 4	5 Als Group 1	5 Als Group 4
Brain	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Spinal cord	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Sciatic nerve	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Stomach	Number of examined sections None	15 100%	15 100%	15 100%	15 100%
Duodenum	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Jejunum	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Ileum	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Caecum	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Colon	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Rectum	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Liver	Number of examined sections Microvacuolation of hepatocytes (areas : 1 and 2; 1 or 2) Micro-and macrovacuolar periportal steatosis (2) Small diffuse granulomae peri centrilobular veins (only certain veins)	15 20% 0% 20% 20%	15 60% 0% 0% 0%	15 80% 20% 40% 40%	15 100% 0% 100% 100%
Spleen	Number of examined sections None	10 100%	10 100%	10 100%	10 100%

Reaction : **0.5** : very slight , **1** : slight, **2** : moderate, **3** : severe

Als : animals

Table 8 (continued)**HISTOPATHOLOGICAL EXAMINATION (% of animals showing abnormalities)**

Organ	Observations	Males		Females	
		5 Als Group 1	5 Als Group 4	5 Als Group 1	5 Als Group 4
Bone marrow (Sternum)	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Mesenteric Lymph Nodes	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Thymus	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Heart	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Trachea	Number of examined sections None	10 100%	10 100%	10 100%	10 100%
Lungs	Number of examined sections None	15 100%	15 100%	15 100%	15 100%
Thyroid / parathyroid	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Adrenal gland (Left)	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Adrenal gland (Right)	Number of examined sections None	5 100%	5 100%	5 100%	5 100%
Kidney (Left)	Number of examined sections Focal interstitial inflammatory reaction with mononuclear cells (1)	15 0%	15 0%	15 20%	15 0%
Kidney (Right)	Number of examined sections None	15 100%	15 100%	15 100%	15 100%
Urinary bladder	Number of examined sections None	5 100%	5 100%	5 100%	5 100%

Reaction : 0.5 : very slight , 1 : slight, 2 : moderate, 3 : severe

Als : animals

Table 8 (continued)**HISTOPATHOLOGICAL EXAMINATION (% of animals showing abnormalities)**

Organ	Observations	Males		Females	
		5 Als Group 1	5 Als Group 4	5 Als Group 1	5 Als Group 4
Testis (Left)	Number of examined sections None	5 100%	5 100%	0	0
Testis (Right)	Number of examined sections None	5 100%	5 100%	0	0
Prostate	Number of examined sections None	5 100%	5 100%	0	0
Ovary (Left)	Number of examined sections None	0	0	5 100%	5 100%
Ovary (Right)	Number of examined sections None	0	0	5 100%	5 100%
Uterus	Number of examined sections None	0	0	25 100%	25 100%

Reaction : 0.5 : very slight , 1 : slight, 2 : moderate, 3 : severe

Als : animals

Appendices

- Appendix 1 : Body weight - Individual values (g)
- Appendix 2 : Food consumption (g/48 hours/ 5 animals' cage)
- Appendix 3 : Haematology - Individual values
- Appendix 4 : Biochemistry – Individual values
- Appendix 5 : Absolute organ weights – Individual values (g)
- Appendix 6 : Relative organ weights (brain weight ratio) - Individual values
- Appendix 7 : Histopathological examination – Individual observations
- Appendix 8 : Control of the concentrations
- Appendix 9 : Food control sheet
- Appendix 10 : Study plan

Appendix 1**BODY WEIGHT - Individual values (g)**

Sex : Male

Animals No	D1	D8	D15	D22	D28	D28-D1
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GROUP 1 10 ml/kg/day, distilled water

9568	266.6	329.3	381.2	421.2	445.1	178.5
9569	278.0	336.2	393.6	446.7	475.8	197.8
9570	266.1	319.6	362.1	404.2	430.4	164.3
9571	268.1	338.6	390.5	440.7	470.0	201.9
9572	241.8	290.3	317.2	351.0	370.5	128.7
Mean	264.1	322.8	368.9	412.8	438.4	174.2
SD	13.4	19.6	31.4	38.4	42.2	29.6

GROUP 2 40 mg/kg/day, ING 911

9578	247.6	307.4	342.2	373.8	400.5	152.9
9579	264.8	323.9	368.5	403.9	429.1	164.3
9580	250.3	311.8	359.5	396.7	426.4	176.1
9581	264.6	335.4	377.0	421.1	455.3	190.7
9582	250.5	318.4	358.0	399.4	424.5	174.0
Mean	255.6	319.4	361.0	399.0	427.2	171.6
SD	8.4	10.9	13.0	17.0	19.5	14.1

GROUP 3 200 mg/kg/day, ING 911

9588	259.7	310.8	357.2	400.0	422.6	162.9
9589	262.6	320.8	371.3	410.7	440.9	178.3
9590	241.7	288.3	317.1	347.2	373.6	131.9
9591	261.0	320.0	363.9	399.1	433.3	172.3
9592	248.9	311.2	361.9	395.9	428.0	179.1
Mean	254.8	310.2	354.3	390.6	419.7	164.9
SD	9.1	13.1	21.4	24.9	26.6	19.6

GROUP 4 1000 mg/kg/day, ING 911

9598	247.3	297.3	334.6	365.9	386.3	139.0
9599	264.0	324.7	357.0	393.2	415.4	151.4
9600	269.4	330.4	387.0	428.2	462.2	192.8
9601	272.4	342.5	387.3	423.8	443.6	171.2
9602	259.2	323.1	371.0	407.3	431.5	172.3
Mean	262.5	323.6	367.4	403.7	427.8	165.3
SD	9.9	16.6	22.2	25.3	28.8	20.8

Appendix 1 (continued)**BODY WEIGHT - Individual values (g)**

Sex : Female

Animals No	D1	D8	D15	D22	D28	D28-D1
GROUP 1 10 ml/kg/day, distilled water						
9573	214.6	237.0	248.2	285.8	300.5	85.9
9574	192.3	222.8	243.2	256.1	267.6	75.3
9575	211.7	229.9	257.8	275.0	282.9	71.2
9576	223.7	239.5	252.5	278.4	288.8	65.1
9577	200.5	225.1	241.2	253.3	251.3	50.8
Mean	208.6	230.9	248.6	269.7	278.2	69.7
SD	12.3	7.3	6.8	14.3	19.2	13.0
GROUP 2 40 mg/kg/day, ING 911						
9583	198.4	214.8	246.2	268.6	266.0	67.6
9584	218.6	240.0	257.6	266.5	282.6	64.0
9585	211.9	228.6	244.5	252.4	269.0	57.1
9586	209.7	233.8	252.4	281.9	285.1	75.4
9587	209.7	244.0	258.4	284.6	290.1	80.4
Mean	209.7	232.2	251.8	270.8	278.6	68.9
SD	7.3	11.4	6.4	13.0	10.5	9.2
GROUP 3 200 mg/kg/day, ING 911						
9593	201.7	220.4	244.9	262.1	261.9	60.2
9594	202.8	207.3	225.9	242.6	244.9	42.1
9595	204.4	218.1	251.6	270.1	271.7	67.3
9596	222.7	239.0	275.7	293.0	299.5	76.8
9597	226.6	258.7	296.7	303.1	311.7	85.1
Mean	211.6	228.7	259.0	274.2	277.9	66.3
SD	12.0	20.3	27.6	24.2	27.4	16.5
GROUP 4 1000 mg/kg/day, ING 911						
9603	220.5	227.5	256.7	271.2	277.7	57.2
9604	226.7	245.3	263.4	279.0	299.2	72.5
9605	219.1	232.8	241.1	259.7	273.4	54.3
9606	199.9	213.2	244.5	261.2	269.8	69.9
9607	190.9	223.1	250.1	268.9	276.9	86.0
Mean	211.4	228.4	251.2	268.0	279.4	68.0
SD	15.2	11.9	9.0	7.9	11.5	12.8

Appendix 2**FOOD CONSUMPTION (g/48 hours/5 animals' cage)**Sex : **Male**

Animals No	Week 1	Week 2	Week 3	Week 4
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GROUP 1 *10 ml/kg/day, distilled water*

9568				
9569				
9570	276	237	256	285
9571				
9572				

GROUP 2 *40 mg/kg/day, ING 911*

9578				
9579				
9580	264	222	285	288
9581				
9582				

GROUP 3 *200 mg/kg/day, ING 911*

9588				
9589				
9590	256	207	270	277
9591				
9592				

GROUP 4 *1000 mg/kg/day, ING 911*

9598				
9599				
9600	262	191	260	268
9601				
9602				

Appendix 2 (continued)**FOOD CONSUMPTION (g/48 hours/5 animals' cage)**

Sex : Female

Animals No	Week 1	Week 2	Week 3	Week 4
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GROUP 1 10 ml/kg/day, distilled water

9573				
9574				
9575	178	191	217	211
9576				
9577				

GROUP 2 40 mg/kg/day, ING 911

9583				
9584				
9585	189	201	237	227
9586				
9587				

GROUP 3 200 mg/kg/day, ING 911

9593				
9594				
9595	178	191	203	203
9596				
9597				

GROUP 4 1000 mg/kg/day, ING 911

9598				
9599				
9600	189	201	193	218
9601				
9602				

Appendix 3**HEMATOLOGY - Abbreviations -**

Eryth	: Erythrocytes
Hb	: Hemoglobin
Ht	: Hematocrit
MCV	: Mean Corpuscular Volume
MCH	: Mean Corpuscular Hemoglobin
MCHC	: Mean Corpuscular Hemoglobin Concentration
Leuko	: Leukocytes
Plat	: Platelets
Prothro. T (s)	: Prothrombine time (seconds)
Ctrl	: Control
N	: Neutrophils
E	: Eosinophils
B	: Basophils
L	: Lymphocytes
M	: Monocytes

Appendix 3 (continued)

HEMATOLOGY - Individual values

Sex : Male

Animals No	Eryth T/l	Hb g/dl	Ht %	MCV Fl	MCH pg	MCHC g/dl	Leuko G/l	Plat. G/l	Prothro. T (s) Ctrl 1	Prothro. T (s) Ctrl 2
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GROUP 1 10 ml/kg/day, distilled water

9568	7.4	17.28	41.6	57	23.35	41.54	17.1	1096	11.7	11.5
9569	7.4	17.92	40.6	57	24.22	44.14	11.4	826	11.1	11.0
9570	6.8	15.02	41.0	64	22.09	36.63	16.3	936	12.7	12.6
9571	7.4	17.37	43.4	60	23.47	40.00	12.7	1028	11.5	11.6
9572	7.4	15.15	42.2	58	20.47	35.90	11.3	872	11.6	11.7
Mean	7.3	16.55	41.8	59	22.72	39.64	13.8	952	11.7	11.7
SD	0.3	1.36	1.1	3	1.47	3.43	2.8	111	0.6	0.6

GROUP 2 40 mg/kg/day, ING 911

9578	7.0	15.23	37.0	54	21.76	41.20	10.0	954	12.1	12.0
9579	6.6	14.13	35.8	56	21.40	39.47	8.5	1064	12.2	12.3
9580	7.0	15.84	41.2	60	22.60	38.46	11.2	1060	11.9	12.0
9581	7.6	16.27	41.0	56	21.40	39.68	10.5	914	12.2	11.9
9582	7.6	16.01	42.6	57	21.07	37.58	8.7	980	12.6	12.3
Mean	7.2	15.50	39.5	57	21.65	39.28	9.8	994	12.2	12.1
SD	0.4	0.85	2.9	2	0.59	1.36	1.2	66	0.3	0.2

GROUP 3 200 mg/kg/day, ING 911

9588	7.4	15.54	38.6	54	21.00	40.26	11.8	990	12.7	12.3
9589	7.2	15.82	40.4	57	21.97	39.16	8.6	1076	12.3	12.0
9590	7.4	15.43	38.8	54	20.85	39.77	8.4	1026	12.7	12.6
9591	7.0	15.70	40.0	58	22.43	39.25	13.7	964	12.6	12.4
9592	7.0	16.30	36.6	55	23.29	44.54	13.3	1056	12.3	12.3
Mean	7.2	15.76	38.9	56	21.91	40.60	11.2	1022	12.5	12.3
SD	0.2	0.34	1.5	2	1.02	2.25	2.5	46	0.2	0.2

GROUP 4 1000 mg/kg/day, ING 911

9598	7.0	15.27	41.6	63	21.81	36.71	12.0	868	19.0	18.6
9599	7.2	15.97	39.4	56	22.18	40.53	11.8	888	12.3	12.0
9600	7.6	16.10	42.0	57	21.18	38.33	8.1	950	12.9	12.8
9601	6.8	14.29	38.2	57	21.01	37.41	8.0	822	12.7	12.8
9602	6.8	15.03	37.4	57	22.10	40.19	8.4	848	12.3	12.4
Mean	7.1	15.33	39.7	58	21.66	38.63	9.7	875	13.8	13.7
SD	0.3	0.74	2.0	3	0.53	1.68	2.1	48	2.9	2.7

Appendix 3 (continued)**HEMATOLOGY - Individual values**Sex : **Male**

Animals No	N %	E %	B %	L %	M %	Total Leuko. G/l	N G/l	E G/l	B G/l	L G/l	M G/l
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GROUP 1 10 ml/kg/day, distilled water

9568	15	1	0	84	0	17.1	2.57	0.17	0.00	14.36	0.00
9569	18	0	0	82	0	11.4	2.05	0.00	0.00	9.35	0.00
9570	10	0	0	88	2	16.3	1.63	0.00	0.00	14.34	0.33
9571	4	0	0	95	1	12.7	0.51	0.00	0.00	12.07	0.13
9572	6	0	0	94	0	11.3	0.68	0.00	0.00	10.62	0.00
Mean	11	0	0	89	1	13.8	1.49	0.03	0.00	12.15	0.09
SD	6	0	0	6	1	2.8	0.88	0.08	0.00	2.23	0.14

GROUP 2 40 mg/kg/day, ING 911

9578	19	2	0	79	0	10.0	1.90	0.20	0.00	7.90	0.00
9579	28	0	0	71	0	8.5	2.38	0.00	0.00	6.04	0.00
9580	21	1	0	78	0	11.2	2.35	0.11	0.00	8.74	0.00
9581	8	1	0	91	0	10.5	0.84	0.11	0.00	9.56	0.00
9582	8	0	0	92	0	8.7	0.70	0.00	0.00	8.00	0.00
Mean	17	1	0	82	0	9.8	1.63	0.08	0.00	8.05	0.00
SD	9	1	0	9	0	1.2	0.81	0.08	0.00	1.31	0.00

GROUP 3 200 mg/kg/day, ING 911

9588	12	0	0	88	0	11.8	1.42	0.00	0.00	10.38	0.29
9589	19	0	0	81	0	8.6	1.63	0.00	0.00	6.97	0.00
9590	8	0	0	92	0	8.4	0.67	0.00	0.00	7.73	0.00
9591	6	0	0	94	0	13.7	0.82	0.00	0.00	12.88	0.00
9592	13	2	0	85	0	13.3	1.73	0.27	0.00	11.31	0.00
Mean	12	0	0	88	0	11.2	1.25	0.05	0.00	9.85	0.06
SD	5	1	0	5	0	2.5	0.48	0.12	0.00	2.47	0.13

GROUP 4 1000 mg/kg/day, ING 911

9598	11	0	0	89	0	12.0	1.32	0.00	0.00	10.68	0.00
9599	12	1	0	87	0	11.8	1.42	0.12	0.00	10.27	0.00
9600	7	0	0	93	0	8.1	0.57	0.00	0.00	7.53	0.00
9601	14	0	0	86	0	8.0	1.12	0.00	0.00	6.88	0.00
9602	15	1	0	84	0	8.4	1.26	0.08	0.00	7.06	0.00
Mean	12	0	0	88	0	9.7	1.14	0.04	0.00	8.48	0.00
SD	3	1	0	3	0	2.1	0.34	0.06	0.00	1.84	0.00

Appendix 3 (continued)**HEMATOLOGY - Individual values**Sex : **Female**

Animals No	Eryth T/l	Hb g/dl	Ht %	MCV Fl	MCH pg	MCHC g/dl	Leuko G/l	Plat. G/l	Prothro. T (s) Ctrl 1	Prothro. T (s) Ctrl 2
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GROUP 1 10 ml/kg/day, distilled water

9573	7.2	13.02	40.8	58	18.08	31.90	12.5	852	11.6	11.5
9574	7.0	14.32	40.0	58	20.46	35.80	10.0	1022	11.7	12.0
9575	6.8	12.21	38.8	58	17.96	31.47	12.0	1100	11.0	10.7
9576	8.2	15.40	44.6	55	18.78	34.53	10.1	814	11.7	11.5
9577	7.2	14.00	38.4	54	19.44	36.46	8.0	826	12.5	12.3
Mean	7.3	13.79	40.5	57	18.94	34.03	10.5	923	11.7	11.6
SD	0.5	1.23	2.5	2	1.04	2.26	1.8	130	0.5	0.6

GROUP 2 40 mg/kg/day, ING 911

9583	7.0	12.51	38.8	57	17.87	32.24	8.7	1108	12.3	12.0
9584	7.4	15.47	40.0	56	20.91	38.68	10.0	916	12.3	12.4
9585	7.4	12.68	42.6	59	17.14	29.77	7.3	1030	11.8	11.9
9586	7.0	12.12	39.0	57	17.31	31.08	10.8	976	12.5	12.3
9587	6.6	13.60	36.6	58	20.61	37.16	9.8	964	11.4	11.4
Mean	7.1	13.28	39.4	57	18.77	33.79	9.3	999	12.1	12.0
SD	0.3	1.34	2.2	1	1.84	3.91	1.4	73	0.5	0.4

GROUP 3 200 mg/kg/day, ING 911

9593	7.2	13.53	39.0	56	18.79	34.69	8.7	840	12.2	12.1
9594	6.6	12.79	36.8	57	19.38	34.76	7.6	948	11.6	12.0
9595	6.6	12.73	36.8	57	19.29	34.59	11.7	964	11.2	11.3
9596	6.8	13.74	39.0	60	20.20	35.23	10.4	860	11.8	12.0
9597	7.0	13.57	37.0	55	19.39	36.68	13.7	1106	11.8	11.8
Mean	6.8	13.27	37.7	57	19.41	35.19	10.4	944	11.7	11.8
SD	0.3	0.47	1.2	2	0.51	0.87	2.4	106	0.4	0.3

GROUP 4 1000 mg/kg/day, ING 911

9603	7.0	13.03	37.8	55	18.61	34.47	8.9	604	13.2	13.2
9604	7.0	13.58	36.2	53	19.40	37.51	10.2	992	11.8	12.0
9605	6.8	13.00	34.0	52	19.12	38.24	8.0	1030	11.9	11.7
9606	7.2	12.29	36.2	52	17.07	33.95	11.0	1018	13.3	13.4
9607	7.2	13.26	37.2	59	18.42	35.65	6.0	750	11.7	12.0
Mean	7.0	13.03	36.3	54	18.52	35.96	8.8	879	12.4	12.5
SD	0.2	0.48	1.4	3	0.90	1.87	2.0	192	0.8	0.8

Appendix 3 (continued)**HEMATOLOGY - Individual values**

Sex : Female

Animals No	N %	E %	B %	L %	M %	Total Leuko. G/l	N G/l	E G/l	B G/l	L G/l	M G/l
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GROUP 1 10 ml/kg/day, distilled water

9573	5	0	0	95	0	12.5	0.63	0.00	0.00	11.88	0.00
9574	7	0	0	92	1	10.0	0.70	0.00	0.00	9.20	0.10
9575	7	1	0	91	1	12.0	0.84	0.12	0.00	10.92	0.12
9576	11	3	0	85	1	10.1	1.11	0.30	0.00	8.59	0.10
9577	5	2	0	92	1	8.0	0.40	0.16	0.00	7.36	0.08
Mean	7	1	0	91	1	10.5	0.74	0.12	0.00	9.59	0.08
SD	2	1	0	4	0	1.8	0.26	0.13	0.00	1.81	0.05

GROUP 2 40 mg/kg/day, ING 911

9583	5	3	0	92	0	8.7	0.44	0.26	0.00	8.00	0.00
9584	3	1	0	96	0	10.0	0.30	0.10	0.00	9.60	0.00
9585	10	1	0	89	0	7.3	0.73	0.07	0.00	6.50	0.00
9586	20	1	0	79	0	10.8	2.16	0.11	0.00	8.53	0.00
9587	10	1	0	89	0	9.8	0.98	0.10	0.00	8.72	0.00
Mean	10	1	0	89	0	9.3	0.92	0.13	0.00	8.27	0.00
SD	7	1	0	6	0	1.4	0.74	0.08	0.00	1.15	0.00

GROUP 3 200 mg/kg/day, ING 911

9593	17	0	0	83	0	8.7	1.48	0.00	0.00	7.22	0.00
9594	16	0	0	84	0	7.6	1.22	0.00	0.00	6.38	0.00
9595	9	0	0	91	0	11.7	1.05	0.00	0.00	10.65	0.00
9596	9	2	0	89	0	10.4	0.94	0.21	0.00	9.26	0.00
9597	11	1	0	88	0	13.7	1.51	0.14	0.00	12.06	0.00
Mean	12	1	0	87	0	10.4	1.24	0.07	0.00	9.11	0.00
SD	4	1	0	3	0	2.4	0.25	0.10	0.00	2.35	0.00

GROUP 4 1000 mg/kg/day, ING 911

9603	9	1	0	89	1	8.9	0.80	0.09	0.00	7.92	0.09
9604	10	0	0	90	0	10.2	1.02	0.00	0.00	9.18	0.00
9605	14	2	0	84	0	8.0	1.12	0.16	0.00	6.72	0.00
9606	12	0	0	88	0	11.0	1.32	0.00	0.00	9.68	0.00
9607	12	1	0	87	0	6.0	0.72	0.06	0.00	5.22	0.00
Mean	11	1	0	88	0	8.8	1.00	0.06	0.00	7.74	0.02
SD	2	1	0	2	0	2.0	0.24	0.07	0.00	1.82	0.04

Appendix 3 (continued)**HEMATOLOGY – Methods**

Parameters	Methods
Leucocyte count (G/l)	COULTER ZM Counter
Erythrocyte count (T/l)	COULTER ZM Counter
Haemoglobin (g/dl)	Spectrophotometer Spectrascan 2800
Mean Corpuscular Volume (fl)	COULTER ZM/Counter Reader VGM/Ht
Platelet count (G/l)	COULTER ZM Counter
Haematocrit (%)	COULTER ZM Counter/Reader VGM/Ht
Total leucocyte count (%)	May – Grünwald – Giemsa Dye (Rhone Poulenc 320311 – 320071 reactives)
Prothrombin Time (sec)	Manual method BioMérieux 68805 reactive

Appendix 3 (continued)**HEMATOLOGY – Methods**

Parameters	Methods
Leukocyte count (G/l)	COULTER ZM Counter
Erythrocyte count (T/l)	COULTER ZM Counter
Hemoglobin (g/dl)	Spectrophotometer Spectrascan 2800
Mean Corpuscular Volume (fl)	COULTER ZM/Counter Reader VGM/Ht
Platelet count (G/l)	COULTER ZM Counter
Hematocrit (%)	COULTER ZM Counter/Reader VGM/Ht
Total leukocyte count (%)	May – Grünwald – Giemsa Dye (Rhone Poulenc 320311 – 320071 reactives)
Prothrombin Time (sec)	Manual method BioMérieux 68805 reactive

Appendix 4 (continued)**BIOCHEMISTRY - Individual values**Sex : **Male**

Animals No	Gluc mmol/l	Crea μmol/l	Urea mmol/l	Chol mmol/l	Prot g/l	Alb g/l
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GROUP 1 10 ml/kg/day, distilled water

9568	5.37	39.2	4.22	1.54	52.1	37.2
9569	6.85	38.9	6.21	1.89	51.7	35.0
9570	6.39	41.5	4.18	1.01	53.4	36.3
9571	5.73	39.2	4.69	1.50	53.1	37.9
9572	6.15	38.2	5.16	1.03	51.1	36.3
Mean	6.10	39.4	4.89	1.39	52.3	36.5
SD	0.57	1.2	0.84	0.37	1.0	1.1

GROUP 2 40 mg/kg/day, ING 911

9578	5.83	37.5	4.61	1.17	48.5	35.7
9579	6.60	37.0	5.39	1.16	53.6	37.1
9580	5.91	43.7	4.73	1.43	51.7	36.4
9581	7.50	37.9	4.12	1.35	54.2	38.3
9582	6.55	39.2	4.07	1.32	50.2	36.1
Mean	6.48	39.1	4.58	1.29	51.6	36.7
SD	0.67	2.7	0.54	0.12	2.4	1.0

GROUP 3 200 mg/kg/day, ING 911

9588	8.19	46.3	7.01	0.92	51.2	35.9
9589	7.51	43.4	7.18	1.44	57.3	39.8
9590	5.32	37.7	4.88	1.08	52.0	36.7
9591	6.09	39.1	3.92	1.41	55.1	38.7
9592	6.98	43.0	4.89	1.30	52.8	36.6
Mean	6.82	41.9	5.58	1.23	53.7	37.5
SD	1.14	3.5	1.44	0.22	2.5	1.6

GROUP 4 1000 mg/kg/day, ING 911

9598	6.98	39.9	6.63	1.13	52.4	36.4
9599	7.55	44.2	6.88	1.19	53.3	36.5
9600	5.39	38.5	4.42	1.31	56.6	38.1
9601	6.80	41.2	4.31	1.46	55.6	37.5
9602	7.54	39.6	4.00	1.21	52.0	37.3
Mean	6.85	40.7	5.25	1.26	54.0	37.2
SD	0.88	2.2	1.39	0.13	2.0	0.7

Appendix 4 (continued)**BIOCHEMISTRY - Individual values**

Sex : Male

Animals No	ASAT UI/l	ALAT UI/l	AP UI/l	Ca mmol/l	Na mmol/l	K mmol/l
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GROUP 1 10 ml/kg/day, distilled water

9568	156	28	187	2.33	135	4.5
9569	114	33	142	2.28	132	4.5
9570	189	45	219	2.29	132	4.2
9571	180	42	211	2.37	134	4.6
9572	141	48	243	2.18	129	4.1
Mean	156	39	200	2.29	132	4.4
SD	30	8	38	0.07	2	0.2

GROUP 2 40 mg/kg/day, ING 911

9578	205	41	212	2.24	136	4.3
9579	203	44	188	2.20	137	4.1
9580	221	46	147	2.29	134	4.5
9581	197	42	141	2.27	131	4.2
9582	306	64	210	2.26	130	4.2
Mean	226	47	180	2.25	134	4.3
SD	45	9	34	0.03	3	0.1

GROUP 3 200 mg/kg/day, ING 911

9588	337	67	257	2.16	135	4.8
9589	172	34	133	2.24	136	4.5
9590	302	61	222	2.30	136	4.4
9591	292	64	145	2.36	132	4.0
9592	354	59	152	2.33	139	4.4
Mean	291	57	182	2.28	136	4.4
SD	71	13	55	0.08	2	0.3

GROUP 4 1000 mg/kg/day, ING 911

9598	258	52	185	2.06	138	4.5
9599	200	46	176	2.15	133	4.2
9600	302	53	205	2.29	133	4.2
9601	313	64	253	2.35	141	4.5
9602	345	66	307	2.33	141	4.2
Mean	284	56	225	2.24	137	4.3
SD	56	8	55	0.13	4	0.1

Appendix 4 (continued)**BIOCHEMISTRY - Individual values**

Sex : Female

Animals No	Gluc mmol/l	Crea μmol/l	Urea mmol/l	Chol mmol/l	Prot g/l	Alb g/l
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GROUP 1 10 ml/kg/day, distilled water

9573	5.29	69.3	8.53	1.36	58.1	41.6
9574	5.57	47.5	8.57	1.54	53.9	37.7
9575	4.29	47.9	8.55	2.00	54.2	35.8
9576	5.98	41.9	6.99	1.17	54.1	39.6
9577	5.89	47.7	4.69	1.29	59.7	41.4
Mean	5.40	50.9	7.47	1.47	56.0	39.2
SD	0.68	10.6	1.69	0.32	2.7	2.5

GROUP 2 40 mg/kg/day, ING 911

9583	4.62	49.6	6.60	2.18	56.3	40.2
9584	5.47	48.8	7.80	1.34	61.7	42.9
9585	5.17	45.7	6.51	1.65	52.5	37.5
9586	5.48	51.5	7.80	1.98	60.3	41.2
9587	5.77	44.2	4.75	1.23	52.6	37.0
Mean	5.30	48.0	6.69	1.68	56.7	39.8
SD	0.44	3.0	1.25	0.41	4.3	2.5

GROUP 3 200 mg/kg/day, ING 911

9593	3.46	52.0	6.27	1.49	53.2	37.1
9594	4.87	52.2	8.26	0.99	54.7	38.5
9595	5.90	46.1	7.88	1.56	59.7	41.6
9596	5.20	47.3	6.39	1.44	54.6	39.1
9597	5.37	46.7	5.86	1.39	54.5	38.7
Mean	4.96	48.9	6.93	1.37	55.3	39.0
SD	0.92	3.0	1.07	0.22	2.5	1.6

GROUP 4 1000 mg/kg/day, ING 911

9603	3.90	54.7	7.73	1.60	55.5	38.8
9604	4.44	48.6	9.41	1.58	55.1	35.9
9605	5.62	40.4	6.73	1.17	65.3	45.1
9606	5.38	51.2	7.03	1.19	51.2	34.9
9607	6.31	47.0	6.36	1.24	51.4	36.2
Mean	5.13	48.4	7.45	1.36	55.7	38.2
SD	0.96	5.3	1.20	0.22	5.7	4.1

Appendix 4 (continued)**BIOCHEMISTRY - Individual values**

Sex : Female

Animals No	ASAT UI/l	ALAT UI/l	AP UI/l	Ca mmol/l	Na mmol/l	K mmol/l
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GROUP 1 10 ml/kg/day, distilled water

9573	112	24	77	2.38	140	4.9
9574	251	53	126	2.11	133	4.5
9575	92	26	96	2.15	134	4.0
9576	206	39	130	2.30	140	4.1
9577	197	38	136	2.37	130	4.1
Mean	172	36	113	2.26	135	4.3
SD	67	12	25	0.13	4	0.4

GROUP 2 40 mg/kg/day, ING 911

9583	124	31	125	2.31	136	4.7
9584	208	37	100	2.25	137	4.0
9585	209	40	68	2.14	130	3.6
9586	266	44	115	2.33	136	4.1
9587	283	50	154	2.23	133	4.2
Mean	218	40	112	2.25	134	4.1
SD	62	7	32	0.07	3	0.4

GROUP 3 200 mg/kg/day, ING 911

9593	136	22	74	2.16	137	7.7
9594	172	30	73	2.16	139	4.8
9595	310	47	108	2.26	134	4.2
9596	733	126	171	2.25	131	3.9
9597	226	42	110	2.33	134	4.9
Mean	338	53	107	2.23	135	5.1
SD	274	42	40	0.07	3	1.5

GROUP 4 1000 mg/kg/day, ING 911

9603	287	49	110	2.17	136	4.6
9604	262	48	58	2.14	144	4.7
9605	210	38	93	2.34	132	4.4
9606	208	37	129	2.09	134	4.4
9607	423	64	106	2.22	127	4.1
Mean	278	47	99	2.19	135	4.4
SD	88	11	26	0.10	6	0.2

Appendix 4 (continued)**BIOCHEMISTRY - Methods**

Parameters	Methods
Glucose (mmol/l)	Colorimetric assay at 505 nm using a coupled enzyme system glucose oxidase/peroxidase on the MERCK-SELECTRA biochemistry analyser BIOTROL DIAGNOSTIC Ref. A02475- BIOMERIEUX Ref. 61270
Creatinine ($\mu\text{mol/l}$)	Colorimetric assay at 505-620 nm using the Jaffé procedure with no deproteinization on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61162
Urea nitrogen (mmol/l)	Enzymatic method in the UV at 340 nm (Urease/glutamate deshydrogenase) on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61975
Total proteins (g/l)	Colorimetric method using the Biuret reaction at 546 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61602
Albumin (g/l)	Colorimetric method at 620 nm using bromocresol green on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61051
Total cholesterol (mmol/l)	Colorimetric assay at 505-620 nm using enzymes cholesterol esterase, cholesterol oxidase and peroxidase on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61218
Aspartate amino transferase (U/l) (ASAT/GOT)	Kinetic assay of GOT activity at 340 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63212
Alanine amino transferase (U/l) (ALAT/GPT)	Kinetic assay of GPT activity at 340 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63312
Alkaline phosphatases (U/l) (AP)	Photometric assay at 405 nm according to the Société Française de Biochimie Clinique (SFBC) on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63657
Calcium (mmol/l)	Dye – binding method using methylthymol blue around 620 nm on the MERCK SELECTRA biochemistry analyser BIOMERIEUX Ref. 61041
Sodium* (mmol/l)	Potentiometric assay using disposable specific electrodes Direct ISE method on Vitros 950 automaton
Potassium* (mmol/l)	Potentiometric assay using disposable specific electrodes Direct ISE method on Vitros 950 automaton

* Assays performed in the Laboratoires RUFFIE- 30 allée de Tourny - 33000 BORDEAUX

Appendix 4 (continued)**BIOCHEMISTRY - Methods**

Parameters	Methods
Glucose (mmol/l)	Colorimetric assay at 505 nm using a coupled enzyme system glucose oxidase/peroxidase on the MERCK-SELECTRA biochemistry analyser BIOTROL DIAGNOSTIC Ref. A02475 - BIOMERIEUX Ref. 61270
Creatinine ($\mu\text{mol/l}$)	Colorimetric assay at 505-620 nm using the Jaffé Procedure with no deproteinization on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61162
Urea (mmol/l)	Enzymatic method in the UV at 340 nm (Urease/glutamate deshydrogenase) on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61975
Total bilirubin ($\mu\text{mol/l}$)	Colorimetric assay at 546 nm of azobilirubin complex formed from total bilirubin and diazotized sulfanilic acid in the presence of DMSO on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61036
Total proteins (g/l)	Colorimetric method using the Biuret reaction at 546 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61602
Albumin (g/l)	Colorimetric method at 620 nm using bromocresol green on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61051
Total cholesterol (mmol/l)	Colorimetric assay at 505-620 nm using enzymes cholesterol esterase, cholesterol oxidase and peroxidase on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61218
Triglycerides (mmol/l)	Colorimetric method at 505-620 nm using lipase/glycerokinase/glycerol 3 phosphate oxidase/peroxidase on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61236
Aspartate amino transferase (U/l) (ASAT/GOT)	Kinetic assay of GOT activity at 340 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63212
Alanine amino transferase (U/l) (ALAT/GPT)	Kinetic assay of GPT activity at 340 nm on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63312
Alkaline phosphatases (U/l) (AP)	Photometric assay at 405 nm according to the Société Française de Biochimie Clinique (SFBC) on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 63657
Phosphorus (mmol/l)	Photometric measurement at 340 nm of the photomolybdenum blue formed by reduction of the molybdophosphate, on the MERCK-SELECTRA biochemistry analyser BIOMERIEUX Ref. 61571
Calcium (mmol/l)	Dye – binding method using methylthymol blue around 620 nm on the MERCK SELECTRA biochemistry analyser BIOMERIEUX Ref. 61041
Sodium* (mmol/l)	Potentiometric assay using disposable specific electrodes Direct ISE method on Vitros 950 automaton
Potassium* (mmol/l)	Potentiometric assay using disposable specific electrodes Direct ISE method on Vitros 950 automaton

* Assays performed in the Laboratoires RUFFIE- 30 allée de Tourny – 33000 BORDEAUX

Appendix 5 (continued)**ABSOLUTE ORGAN WEIGHTS - Individual values (g)**

Sex : Female

Animals No	Brain	Adrenal		Spleen	Liver	Kidney		Thymus	Heart
		Left	Right			Left	Right		

GROUP 1 10 ml/kg/day, distilled water

9573	1.983	0.035	0.032	0.704	7.600	0.935	0.925	0.454	1.028
9574	1.950	0.028	0.028	0.370	6.670	0.792	0.782	0.443	0.847
9575	1.942	0.044	0.041	0.550	7.640	1.008	0.955	0.624	0.934
9576	1.902	0.029	0.021	0.491	7.171	0.888	0.895	0.364	0.972
9577	1.893	0.039	0.035	0.382	6.667	0.785	0.764	0.286	0.941
Mean	1.934	0.035	0.031	0.499	7.150	0.882	0.864	0.434	0.944
SD	0.037	0.007	0.008	0.137	0.476	0.095	0.086	0.126	0.066

GROUP 2 40 mg/kg/day, ING 911

9583	1.700	0.034	0.040	0.476	6.892	0.880	0.857	0.434	0.916
9584	1.955	0.039	0.041	0.426	7.034	0.859	0.855	0.561	0.943
9585	2.031	0.036	0.032	0.508	7.117	0.843	0.881	0.622	0.936
9586	2.005	0.030	0.032	0.664	7.282	0.944	0.938	0.537	0.935
9587	1.997	0.038	0.033	0.610	7.264	0.920	0.950	0.482	0.948
Mean	1.938	0.035	0.036	0.537	7.118	0.889	0.896	0.527	0.936
SD	0.136	0.004	0.005	0.098	0.163	0.042	0.045	0.072	0.012

GROUP 3 200 mg/kg/day, ING 911

9593	2.002	0.039	0.034	0.475	6.162	0.904	0.860	0.355	0.862
9594	1.871	0.031	0.028	0.426	6.029	0.753	0.794	0.371	0.770
9595	2.010	0.036	0.038	0.456	7.133	0.880	0.891	0.500	0.997
9596	2.059	0.041	0.044	0.550	8.628	1.108	1.133	0.330	0.940
9597	2.082	0.039	0.033	0.737	9.054	1.012	0.993	0.707	0.964
Mean	2.005	0.037	0.035	0.529	7.401	0.931	0.934	0.453	0.907
SD	0.082	0.004	0.006	0.125	1.390	0.135	0.132	0.157	0.091

GROUP 4 1000 mg/kg/day, ING 911

9603	1.954	0.034	0.037	0.528	7.303	0.841	0.912	0.512	0.911
9604	1.886	0.034	0.030	0.707	7.666	0.889	0.878	0.587	1.006
9605	2.023	0.035	0.022	0.389	7.122	0.853	0.889	0.518	0.999
9606	1.943	0.032	0.032	0.529	6.805	0.854	0.873	0.644	0.813
9607	1.959	0.037	0.037	0.591	7.272	0.858	0.858	0.389	1.087
Mean	1.953	0.034	0.032	0.549	7.234	0.859	0.882	0.530	0.963
SD	0.049	0.002	0.006	0.115	0.312	0.018	0.020	0.096	0.105

Appendix 6**RELATIVE ORGAN WEIGHTS (brain weight ratio)
Individual values**

Sex : Male

Animals No	Adrenal		Spleen	Liver	Kidney		Thymus	Heart	Testis		Epididymis	
	Left	Right			Left	Right			Left	Right	Left	Right

GROUP 1 10 ml/kg/day, distilled water

9568	1.20	1.15	42.84	547.65	75.05	73.95	21.29	73.70	73.10	71.29	23.15	22.04
9569	1.90	1.79	39.69	701.79	82.87	84.46	24.41	73.38	74.72	66.92	28.46	29.59
9570	1.52	1.42	37.91	588.01	72.46	70.02	34.10	63.77	77.34	76.78	27.13	28.61
9571	1.20	1.10	31.26	608.13	73.83	73.58	29.66	70.59	72.98	74.33	21.73	22.93
9572	1.35	1.35	32.88	474.29	66.07	67.86	19.23	64.82	75.54	76.93	29.20	27.40
Mean	1.43	1.36	36.92	583.97	74.06	73.97	25.74	69.25	74.73	73.25	25.93	26.12
SD	0.29	0.28	4.80	83.41	6.02	6.39	6.10	4.70	1.81	4.21	3.31	3.42

GROUP 2 40 mg/kg/day, ING 911

9578	1.14	1.14	32.11	470.62	62.38	59.70	22.03	59.16	81.24	79.65	22.98	22.53
9579	1.15	1.15	27.73	558.05	73.14	73.19	21.43	62.18	74.44	75.30	26.19	25.56
9580	1.29	1.39	34.83	587.43	70.89	73.47	27.25	78.83	68.01	72.44	21.64	22.15
9581	1.39	1.49	36.21	578.32	70.06	72.47	27.62	65.13	77.21	73.82	25.02	26.97
9582	1.33	1.33	32.11	500.62	67.03	62.66	19.90	59.76	74.30	75.25	20.00	22.61
Mean	1.26	1.30	32.60	539.01	68.70	68.30	23.65	65.01	75.04	75.29	23.17	23.97
SD	0.11	0.15	3.25	51.00	4.15	6.59	3.55	8.07	4.84	2.71	2.50	2.17

GROUP 3 200 mg/kg/day, ING 911

9588	1.20	1.25	32.13	504.81	66.57	67.50	22.28	62.46	72.45	76.47	22.19	21.96
9589	1.25	1.35	31.76	645.41	69.95	68.60	22.42	66.48	79.45	81.42	26.67	27.66
9590	1.26	1.26	28.29	474.82	68.69	66.80	23.09	59.89	76.47	75.60	26.01	28.73
9591	1.37	1.27	34.20	587.91	72.15	71.65	22.36	71.65	82.98	83.03	29.12	29.98
9592	1.80	1.66	31.56	578.76	80.37	74.87	26.79	64.30	81.78	83.88	26.98	28.49
Mean	1.38	1.36	31.59	558.34	71.55	69.88	23.39	64.95	78.63	80.08	26.19	27.37
SD	0.25	0.17	2.12	68.40	5.33	3.35	1.93	4.45	4.26	3.81	2.52	3.13

GROUP 4 1000 mg/kg/day, ING 911

9598	1.33	1.13	29.22	496.70	70.14	68.77	23.17	60.40	71.72	70.04	23.17	22.43
9599	1.30	1.25	34.57	556.90	77.56	83.34	34.77	68.40	79.07	82.82	25.82	28.47
9600	1.51	1.46	38.74	623.73	81.09	81.92	25.83	71.88	79.04	80.21	24.71	23.88
9601	1.15	1.10	35.93	614.11	68.37	66.51	23.44	68.42	87.75	87.46	23.06	24.64
9602	1.15	1.01	35.65	521.79	69.65	71.81	23.40	63.01	74.48	73.98	20.73	21.28
Mean	1.29	1.19	34.82	562.65	73.36	74.47	26.12	66.42	78.41	78.90	23.50	24.14
SD	0.15	0.17	3.49	55.75	5.62	7.70	4.96	4.63	6.09	6.95	1.93	2.75

*: value non considered for mean calculation

Appendix 6 (continued)**RELATIVE ORGAN WEIGHTS (brain weight ratio)
Individual values**

Sex : Female

Animals No	Adrenal		Spleen	Liver	Kidney		Thymus	Heart
	Left	Right			Left	Right		

GROUP 1 10 ml/kg/day, distilled water

9573	1.77	1.61	35.50	383.26	47.15	46.65	22.89	51.84
9574	1.44	1.44	18.97	342.05	40.62	40.10	22.72	43.44
9575	2.27	2.11	28.32	393.41	51.91	49.18	32.13	48.09
9576	1.52	1.10	25.81	377.02	46.69	47.06	19.14	51.10
9577	2.06	1.85	20.18	352.19	41.47	40.36	15.11	49.71
Mean	1.81	1.62	25.76	369.59	45.57	44.67	22.40	48.84
SD	0.35	0.39	6.68	21.62	4.62	4.16	6.30	3.34

GROUP 2 40 mg/kg/day, ING 911

9583	2.00	2.35	28.00	405.41	51.76	50.41	25.53	53.88
9584	1.99	2.10	21.79	359.80	43.94	43.73	28.70	48.24
9585	1.77	1.58	25.01	350.42	41.51	43.38	30.63	46.09
9586	1.50	1.60	33.12	363.19	47.08	46.78	26.78	46.63
9587	1.90	1.65	30.55	363.75	46.07	47.57	24.14	47.47
Mean	1.83	1.85	27.69	368.51	46.07	46.38	27.15	48.46
SD	0.21	0.35	4.46	21.31	3.83	2.91	2.56	3.14

GROUP 3 200 mg/kg/day, ING 911

9593	1.95	1.70	23.73	307.79	45.15	42.96	17.73	43.06
9594	1.66	1.50	22.77	322.23	40.25	42.44	19.83	41.15
9595	1.79	1.89	22.69	354.88	43.78	44.33	24.88	49.60
9596	1.99	2.14	26.71	419.04	53.81	55.03	16.03	45.65
9597	1.87	1.59	35.40	434.87	48.61	47.69	33.96	46.30
Mean	1.85	1.76	26.26	367.76	46.32	46.49	22.48	45.15
SD	0.13	0.26	5.36	56.94	5.15	5.19	7.22	3.23

GROUP 4 1000 mg/kg/day, ING 911

9603	1.74	1.89	27.02	373.75	43.04	46.67	26.20	46.62
9604	1.80	1.59	37.49	406.47	47.14	46.55	31.12	53.34
9605	1.73	1.09	19.23	352.05	42.17	43.94	25.61	49.38
9606	1.65	1.65	27.23	350.23	43.95	44.93	33.14	41.84
9607	1.89	1.89	30.17	371.21	43.80	43.80	19.86	55.49
Mean	1.76	1.62	28.23	370.74	44.02	45.18	27.19	49.33
SD	0.09	0.33	6.58	22.67	1.88	1.38	5.20	5.42

Appendix 7**HISTOPATHOLOGICAL EXAMINATION - Individual observations**
- Anomalies -Sex : **Male**

Animals No	Organ	Noted anomalies
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GROUP 1 10 ml/kg/day, distilled water

9570	Liver	Microvacuolation of hepatocytes (areas : 1 and 2) (1)
9571	Liver	Many small granulomae peri-centrilobuar veins

GROUP 4 1000 mg/kg/day, ING 911

9598	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2)
9599	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (1)
9602	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2)

Reaction : **0.5** : very slight, **1** : slight, **2** : moderate, **3** : severe

Appendix 7 (suite)**HISTOPATHOLOGICAL EXAMINATION - Individual observations**
- Anomalies -**Sex : Female**

Animals No	Organ	Noted anomalies
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GROUP 1 10 ml/kg/day, distilled water

9573	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2) Some small diffuse granulomae pericentrilobular veins
9574	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2)
9575	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2) Some small diffuse granulomae pericentrilobular veins
9576	Liver	Micro and macrovacuolar periportal steatosis (2)
9577	Liver Kidney (left)	Microvacuolation of hepatocytes (areas 1 and 2) (1) Focal interstitial inflammatory reaction with mononuclear cells (1)

GROUP 4 1000 mg/kg/day, ING 911

9603	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2)
9604	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (1) Small granulomae peri-centrilobular veins and diffuse into the liver parenchyma (some)
9605	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2) Some small diffuse granulomae peri-centrilobular veins
9606	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (1)
9607	Liver	Microvacuolation of hepatocytes (areas 1 and 2) (2)

Reaction : 0.5 : very slight, 1 : slight, 2 : moderate, 3 : severe

Appendix 8
Control of the concentrations

Preparation of the 08/07/03 (**D1**)

Group	ING 911 (mg/kg/day)	Theoretical [ING 911] (mg/ml)	Theoretical percentage of bioactive peptide	Percentage of bioactive peptide measured	% of variation % measured/%theoretical
2	40	4	2.64	2.55	- 3.4
3	200	20	2.64	2.80	+ 6.1
4	1000	100	2.64	2.79	+ 5.7

Preparation of the 22/07/03 (**D15**)

Group	ING 911 (mg/kg/day)	Theoretical [ING 911] (mg/ml)	Theoretical percentage of bioactive peptide	Percentage of bioactive peptide measured	% of variation % measured/%theoretical
2	40	4	2.64	2.61	- 1.1
3	200	20	2.64	2.60	- 1.5
4	1000	100	2.64	2.68	+ 1.5

Preparation of the 04/08/03 (**D28**)

Group	ING 911 (mg/kg/day)	Theoretical [ING 911] (mg/ml)	Theoretical percentage of bioactive peptide	Percentage of bioactive peptide measured	% of variation % measured/%theoretical
2	40	4	2.64	2.53	-4.2
3	200	20	2.64	2.64	0
4	1000	100	2.64	2.45	- 7.2

CONFIDENTIAL**Chromatographic analysis and quantity determination of the bioactive peptide
in the hydrolysate PRODIET F 200 products range**

A detection, characterization and quantitative determination protocol of the $\alpha_{s1}CN$ (f91-100) peptide has been designed by Ingredia and the «Laboratoire des Biosciences de l'Aliment» of Nancy (France). This HPLC protocol applied to the hydrolysate offers an evaluation of the bioactive peptide quantity in the PRODIET F 200 (> 1.7% w/w), and the control of the process reproducibility.

Detection and $\alpha_{s1}CN$ (f91-100) peptide characterization

The spray-dried hydrolysate powder is dissolved at a 2 mg/mL concentration in a 75/25 mixing water / acetonitrile, containing 0,1% (v/v) of trifluoroacetic acid. After dissolution during 30 min under stirring, the solution is filtered on PVDF filter (0.45 μ m). The constituents separation is realized by high performance liquid chromatography on a reversed phase column C18 XTerraTM (4.6 x 250 mm, particles size 5 μ m, porosity 10 nm) (Waters, Milford, MA, USA) maintained at 37°C. The column is connected to an Alliance 2690 system (Waters). The elution is realized using a gradient of 25% B in A to 37% B in A, during 30 minutes, at a 0.8 mL/min flow rate.

A: water + 0.1 % (v/v) trifluoroacetic acid

B: acetonitrile + 0.1% (V/V) trifluoroacetic acid.

The sample volume injected is 100 μ L. In such conditions, the CN α_{s1} -f (91-100) peptide is eluted with a retention time of 20.1 +/- 0.4 min, it means an acetonitrile concentration of about 32% (taking into account the dead volume). The peptide can be detected with an UV monitor between 210 and 290 nm wavelengths (a double wavelength detection is recommended).

The peptide absorption is characteristic between 240 and 295 nm due to tyrosine residues. A ratio A_{215}/A_{280} of 6.7 +/-0.2 and its spectrum between 210 and 290nm are particularly relevant to identify it.

The peptide quantification should be done at 215 nm, with the same analysis protocol as above.

CONFIDENTIAL

$\alpha_{s1}CN$ (f91-100) peptide purity control

It can be directly done in previously described conditions, but a calibration with pure peptide must be previously realized and allows to link the peptide quantity with the AUC (area under the curve) under the peptide peak.

Standard calibration

The pure $\alpha_{s1}CN$ (f91-100) peptide is dissolved in a mixing water/acetonitrile : 71/29 containing 0.1 % (v/v) of trifluoroacetic acid. After dissolution, the solution is filtered on PVDF filter 0.45 µm. A reversed phase column LichroCart C18 (100 RP-18 endcapped, dimensions: 4 x 250 mm, particles size: 5 µm, porosity: 10 nm) is used. The gradient increases from 29 % B in A to 39 % B in A during 20 minutes, at a 1 mL/min flow rate.

A: water + 0.1 % (v/v) trifluoroacetic acid

B: acetonitrile + 0.1% (v/v) trifluoroacetic acid.

Five successive analyses will be done in applying samples of 5, 10, 20, 40 and 80 µl of the pure peptide solution. UV detection is realized at 280 nm, with an UV monitor. The standard curve "quantity = f(A₂₈₀)" will allow, during an analysis, the determination of the peptide quantity depending on the registered absorbance at a 280 nm wavelength.

M

Tj 289/03-2070

RepF200 Report ⁵⁶

Reported by User: System

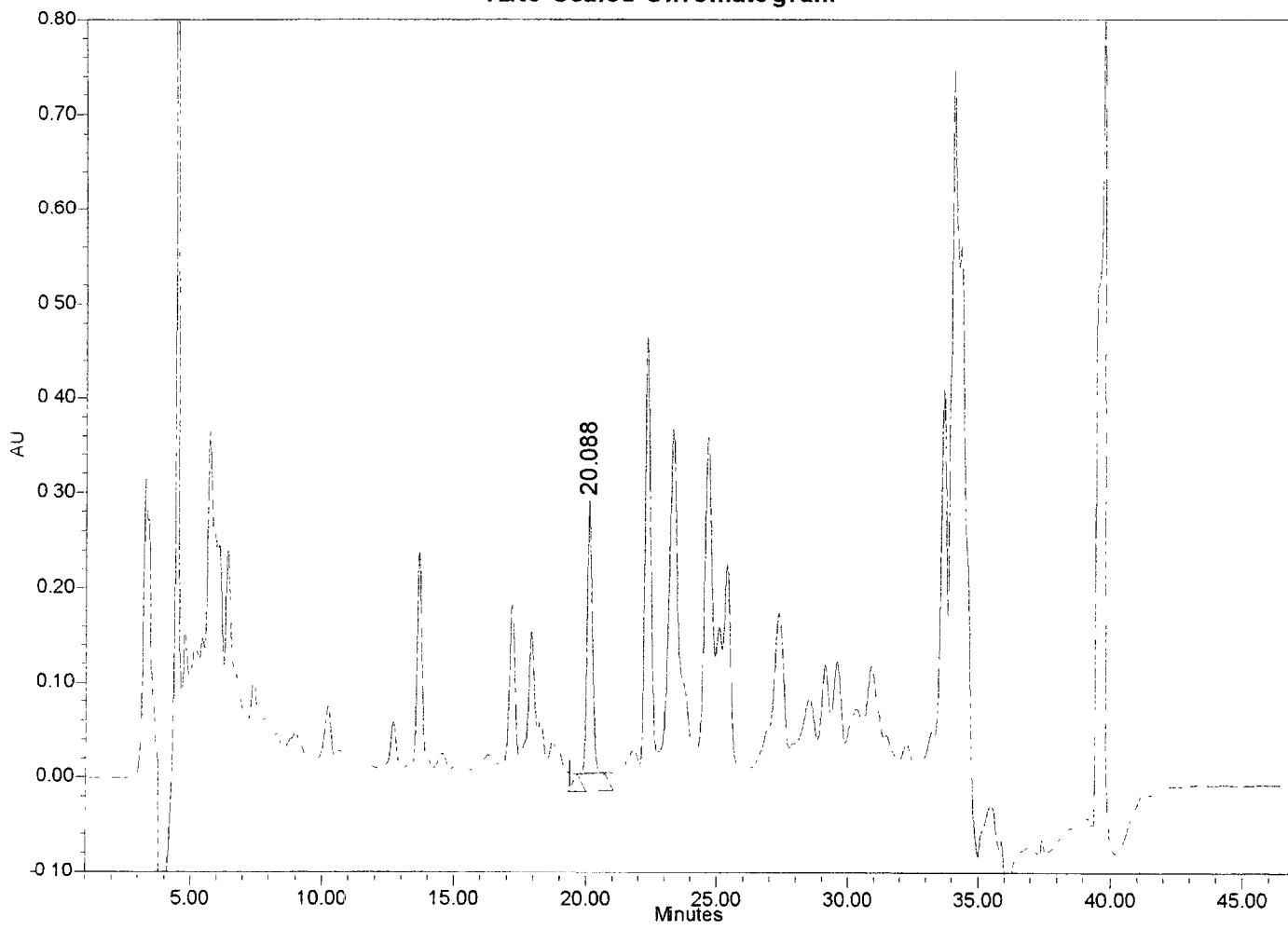
Project Name: C18

SAMPLE INFORMATION

Sample Name: Evi c ING 911 lot 2
Sample Type: Unknown
Vial: 3
Injection #: 1
Injection Volume: 100.00 ul
Run Time: 47.0 Minutes
Sample Set Name: Essai 27

Acquired By: System
Date Acquired: 15/07/03 15:36:32
Acq. Method Set: Set F200
Date Processed: 15/07/03 16:23:44
Processing Method: f200c
Channel Name: extrait 215
Proc. Chnl. Descr. PDA 215.0 nm

Auto-Scaled Chromatogram



Peak Results

Name	RT	Area	Height	Amount	Units
f91-100	19.350				
	20.088	3960139	274683		

3,35%

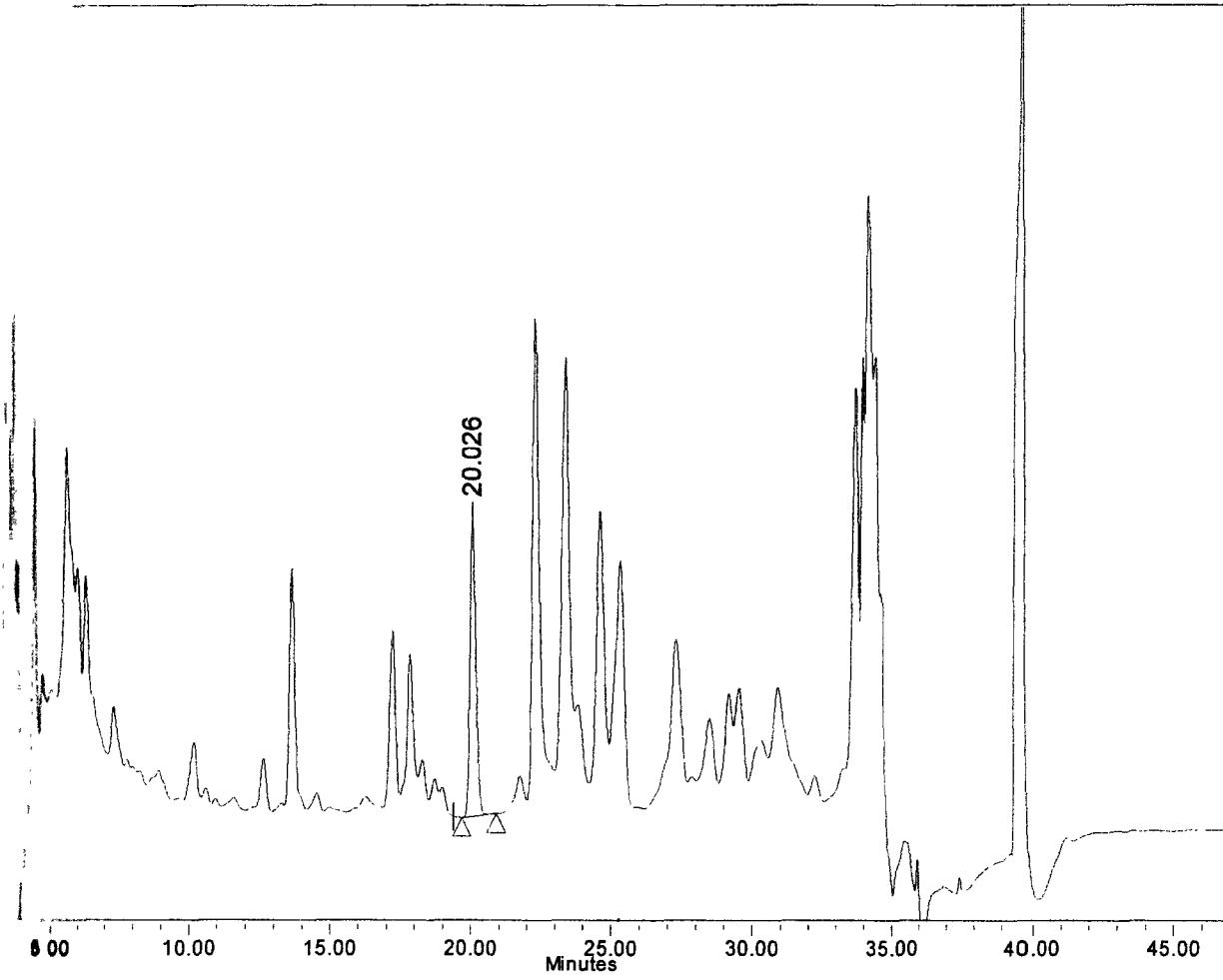
Reported by User: System

Project Name: C18

SAMPLE INFORMATION

Name: Evic ING 911 lot 3
 Type: Unknown
 4
 1
 Volume: 100.00 ul
 47.0 Minutes
 Date Name: Essai 27

Acquired By: System
 Date Acquired: 15/07/03 16:25:05
 Acq. Method Set: Set F200
 Date Processed: 15/07/03 17:12:16
 Processing Method f200c
 Channel Name: extrait 215
 Proc. Chnl. Descr. PDA 215.0 nm

Auto-Scaled Chromatogram**Peak Results**

#	Area	Height	Amount	Units
360				
026	4339489	295971		

2,801

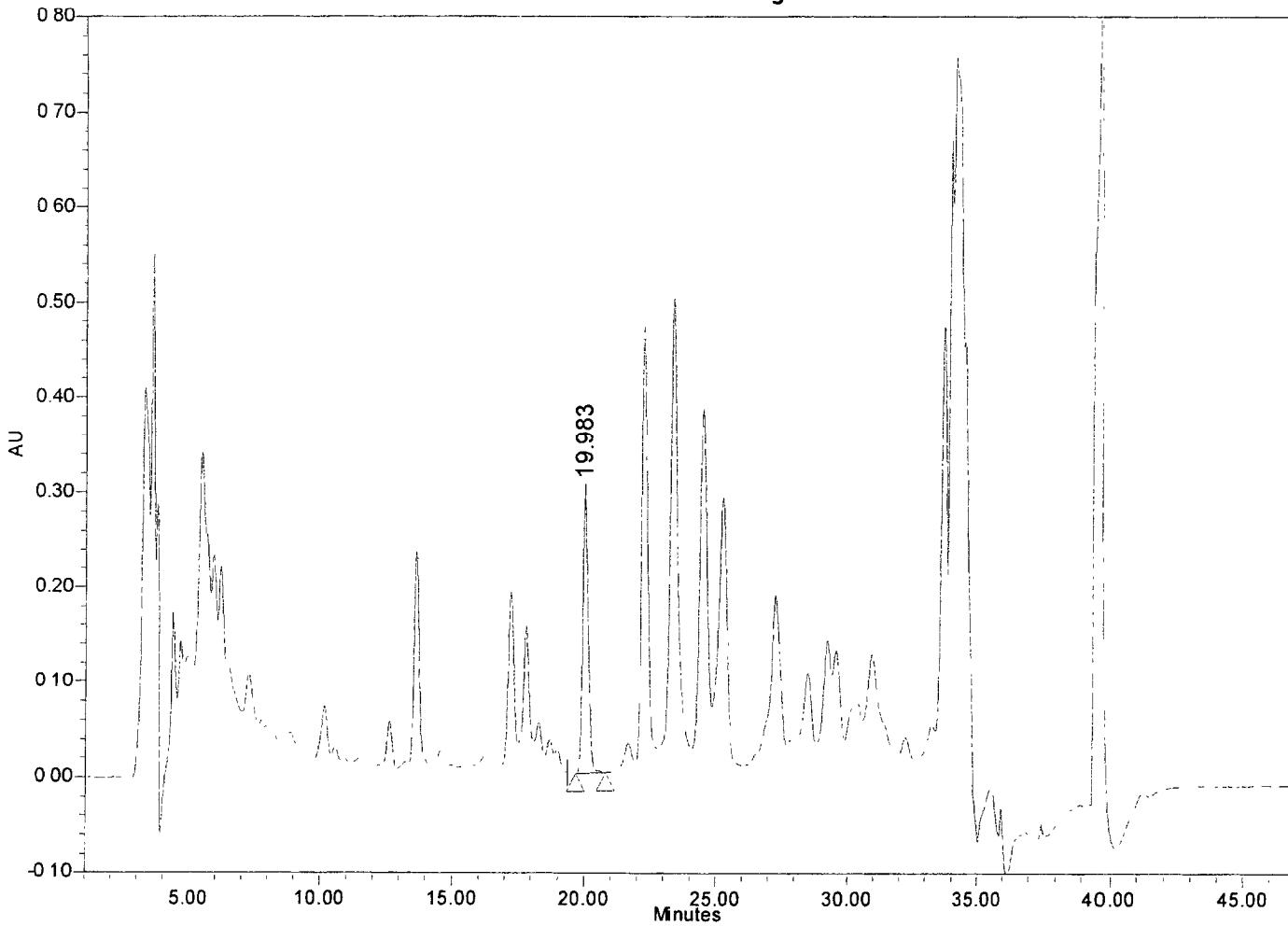
Reported by User: System

Project Name: C18

SAMPLE INFORMATION

Sample Name:	EviC ING 911 lot 4	Acquired By :	System
Sample Type:	Unknown	Date Acquired:	15/07/03 17:13:35
Vial:	5	Acq. Method Set:	Set F200
Injection #:	1	Date Processed:	15/07/03 18:00:47
Injection Volume:	100.00 ul	Processing Method:	f200c
Run Time:	47.0 Minutes	Channel Name:	extrait 215
Sample Set Name:	Essai 27	Proc. Chnl. Descr.	PDA 215.0 nm

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	f91-100	19.350				
2		19.983	4316865	292233		

2,771

Appendix 9

Food control sheet



FICHE CONTROLE

A04C-10 lot 30130

Scientific Animal Food & Engineering
Date de Fabrication

30/01/2003

Date limite de vente 30/05/2003

Date limite d'utilisation 30/01/2004

Numéros des sacs : 1450 à 2000

Quantité fabriquée (en tonnes) 40
Contrôle de la composition centésimale Conforme

TECHNOLOGIE DES PELLETS

Diamètre	(en mm)	16.04	±	0.10	(15.5 à 17.0)
Résistance à l'écrasement	(en Kgf / cm²)	20.2	±	1.6	(15 à 30)
Résistance à l'abrasion	(en %)	98.6			(> 97)
Masse Spécifique	(en g / l)	689			
Poids	(en g)	4.389	±	0.510	
Longueur	(en mm)	20.8	±	1.6	(18.0 à 26.0)
Longueur < Diamètre.....	(en %)	0.2			(< 2)
Nombre de pellets chauffés par Kg	(/ Kg)	0			(< 1)

CONTROLE DE LA QUALITE NUTRITIVE

Témoin incorporation mélange minéral	(Na)	Positif	
Témoin incorporation pre-mélange oligo-éléments (Mn et Cu)		Positif	
Témoin incorporation pré-mélange vitamines .. (Vit.A et E)		Positif	
Eau	(en %)	13.0	(9 à 14)
Protéines	(en %)	15.2	(14.5 à 18.0)
Lipides	(en %)	3.1	(1.7 à 3.7)
Glucides E.N.A.	(en %)	60.3	(57.0 à 63.0)
Dont Amidon	(en %)	45.0	(35.0 à 53.0)
" Sucres totaux	(en %)	3.1	
Cellulose WEENDE	(en %)	3.7	(3.0 à 5.5)
Hémicellulose	(en %)		
Cellulose vraie	(en %)		
Lignine	(en %)		
Minéraux totaux	(en %)	4.7	(4.0 à 6.0)
Dont Calcium	(en mg / Kg)	6 800	(6 000 à 10 000)
" Phosphore	(en mg / Kg)	5 300	(4 500 à 7 000)
" Sodium	(en mg / Kg)	2 900	(1 500 à 3 500)
" Potassium	(en mg / Kg)	6 700	(5 500 à 8 500)
" Manganèse	(en mg / Kg)	61	(40 à 100)
" Cuivre	(en mg / Kg)	18	(10 à 35)
" Vitamine A	(en UI / Kg)	5 400	(4 000 à 11 000)
" Vitamine C	(en mg / Kg)		
" Vitamine E	(en mg / Kg)	30	

CONTROLE DES CONTAMINANTS

BACTERIOLOGIQUES

Microorganismes aérobies 30° C ... (/g)	< 100	(< 100 000)	
Moississures & levures (/g)	< 10	(< 1 000)	
Coliformes 30° C (/g)	0	(< 5)	
Coliformes thermotolérants (/g)	0	(0)	
Anaérobies sulfito-réductrices 46° C (/g)	< 10	(< 100)	
Salmonelles (/25 g)	0	(0)	

MYCOTOXIQUES (en µg / Kg)

Aflatoxines	< 1	(< 5)
Risque mycotoxique global	Négatif	
Observations éventuelles		

<u>METAUX LOURDS</u>		<u>DERIVES NITROSES</u>	
Plomb	(en µg / Kg)	20	(< 1 500)
Mercure	(en µg / Kg)	21	(< 100)
Arsenic	(en µg / Kg)	72	(< 1 000)
Cadmium	(en µg / Kg)	24	(< 250)
Sélénium ..	(en µg / Kg)	170	(< 600)
<u>PESTICIDES ORGANOS-CHLORES (en µg / Kg)</u>		(Total < 200)	
Lindane		< 1	(< 100)
a HCH		< 1	(< 20)
b HCH		< 5	(< 10)
d HCH		< 5	(< 100)
HCB		< 1	(< 10)
PCB		< 50	(< 50)
Aldrine		< 1	(< 10)
Dieldrine		< 1	(< 20)
Endosulfan		< 1	(< 100)
<u>PESTICIDES ORGANOS-PHOSPHORES (en µg / Kg)</u>		(Total < 7 000)	
Acéphate		< 500	(< 5 000)
Azinphos éthyl		< 50	(< 5 000)
Azinphos méthyl		< 50	(< 5 000)
Bromophos éthyl		< 10	(< 5 000)
Bromophos méthyl		< 20	(< 5 000)
Carbophénothion éthyl		< 50	(< 5 000)
Carbophénothion méthyl		< 20	(< 5 000)
Chlorfenvinphos		< 10	(< 5 000)
Chlorméphos		< 10	(< 5 000)
Chlorpyriphos éthyl		< 15	(< 5 000)
Chlorpyriphos méthyl		< 15	(< 1 500)
Chlorthiofos		< 15	(< 5 000)
Iazinon		< 15	(< 5 000)
Chlوفenthion		< 10	(< 5 000)
Chlorvos		< 20	(< 5 000)
Éthion		< 10	(< 5 000)
Iméfox		< 20	(< 5 000)
Iméthoate		< 30	(< 1 000)
Isoxathion		< 15	(< 5 000)
Sulfoton		< 30	(< 5 000)
Hoprophos		< 20	(< 5 000)
Enchlorphos		< 20	(< 5 000)
Nitrothion		< 15	(< 5 000)
Orthion		< 30	(< 5 000)
nofos		< 20	(< 5 000)
rmothion		< 20	(< 5 000)
pténophos		< 30	(< 5 000)
<u>YRETHRINOIDES DE SYNTHESE (en µg / Kg)</u>		ND	ND
.....		ND	ND

EMARQUES

oratoire Contrôle AQ
Le Responsable

11/03/2003

Le Responsable AQ

Appendix 10

Study plan



EXEMPLAIRE A NOUS RETOURNER
SIGNÉ S.V.P.

INGREDIA

51-53 Avenue de Lobbedez
BP 946
62033 ARRAS

A l'attention de Mme Catherine LEFRANC

N/Réf. : PhD/VM – PSp. 03-0444/1

Etude : Tj 289

Blanquefort, le 4 Juillet 2003

**EVALUATION DE LA TOXICITE PAR ADMINISTRATION ORALE REPETEE
PENDANT 28 JOURS CHEZ LE RAT D'UN COMPLEMENT ALIMENTAIRE****- PROTOCOLE -****1. OBJECTIF ET PRINCIPE DE L'ESSAI**

L'objectif de l'essai est d'apprecier qualitativement et quantitativement les phénomènes toxiques et leur délai d'apparition après administration répétée, par voie orale chez le Rat, du produit ING 911 (hydrolysat de caseine α S1) utilisé comme complément alimentaire.

La préparation à tester est administrée quotidiennement pendant 28 jours consécutifs au moins, par gavage, à des doses croissantes, à plusieurs lots d'animaux (lots traités) à raison d'une dose par lot. Parallèlement un groupe d'animaux (lot témoin) reçoit pendant 28 jours consécutifs le même volume de véhicule dans les mêmes conditions.

Pendant la période d'administration, les animaux sont observés chaque jour, pour déceler les éventuels signes de toxicité. Des examens biochimiques et hématologiques sont réalisés en fin de traitement sur tous les animaux.

Tous les animaux utilisés dans l'essai sont autopsiés et soumis à des examens histopathologiques appropriés.

Le Rat est l'espèce rongeur communément utilisée et recommandée par les autorités officielles pour l'évaluation de la sécurité des substances chimiques et médicamenteuses, par ce type d'essai.

La méthodologie utilisée suit les lignes directrices n° 407 de l'OCDE du 27 juillet 1995 et l'Annexe IV.D section B7 de la Directive Européenne 96/54/CEE du 30 juillet 1996 publiée au Journal Officiel de Communautés Européennes du 30 septembre 1996 (L248).

2. INSTALLATION D'ESSAI ET INTERVENANTS

2.1. Installation d'Essai et équipe technique

EVIC France – Division Evic-Tox
48 rue Jean Duvert
33290 Blanquefort
05 56 95 59 95

Directeur de l'étude : Philippe DUFOUR
Technicien responsable : Martine MIERMON

GREF/INSERM E9917
Université Victor Segalen – Bordeaux 2
146 rue Léo Saignat
33076 Bordeaux Cedex
05 57 57 17 71

Anatomopathologiste : Rosa URBANIAK

2.2. Intervenants

Analyses biochimiques (Na/K)

Laboratoire d'Analyses de Biologie Médicale Ruffié et Associés Biogiste : Ch. DUBOIS
17 allées de Tourny
33080 Bordeaux Cedex
05 56 79 45 00

Analyses hématologiques et biochimiques

EVIC France – Division Evic-Bio
48 rue Jean Duvert
33290 Blanquefort
05 56 95 59 95

Responsable : M.A. ALONSO

2.3. Ethique et agréments de l'Installation d'Essai

L'étude est entièrement réalisée selon les règles d'éthique animale indiquées dans la Directive Européenne 86/609/CEE du 24 novembre 1986 et est soumise à l'avis préalable du Comité d'Ethique Animale interne à l'Installation d'Essai.

Elle est conduite selon les règles internes du Système Qualité d'EVIC france, qui a été reconnu conforme aux BPL par l'**AFSSAPS** (arrêté du 14 mars 2000 publié au JORF du 23 mars 2000) et le **GIPC** (décret n° 98-1312 du 31 décembre 1998 publié au JORF du 1er janvier 1999) et à la norme NF EN ISO/CEI 17025 par le **COFRAC** (accréditation n°1-0042).

3. PRODUIT A TESTER

Dénomination : ING 911

Date de réception : 25 juin 2003

Caractères organoleptiques : poudre blanche

Quantité, conditionnement : 500 g, sachet aluminium thermosoudé

Référence dans l'Installation d'Essai : 03-2070

Stockage : dans son conditionnement d'envoi

Echantillonnage : un échantillon de la préparation est archivé dans l'échantillothèque de l'Installation d'Essai et est conservé jusqu'à sa date de péremption ou pendant une durée de 10 ans maximum.

4. SYSTEME REACTIF

Espèce : Rats albinos EOPS (exempts d'organisme pathogène spécifique) Sprague-Dawley

Origine : CHARLES RIVER Laboratories (69592 L'Arbresle cedex, France)

Age : entre 5 et 6 semaines (à la mise en acclimatation)

Nombre et sexe : 40 (20 mâles et 20 femelles nullipares et non gravides) répartis en 4 lots de 10 animaux (5 mâles et 5 femelles).

Acclimatation : pendant 5 jours au moins avant le début de l'essai

Répartition des animaux par lot :

- *Pesée* : pendant la période d'acclimatation (J-1), les animaux sont pesés. Les animaux sont répartis en lots traités et lot témoin par tirage au sort, selon la table de randomisation de Moses et Oakford. Un contrôle de l'homogénéité des lots est effectué par analyse des coefficients de variation après répartition, sur la base du poids corporel. Pour chaque groupe (mâles ou femelles), le poids moyen est calculé et les limites acceptables sont déduites, les poids individuels extrêmes des animaux ne devant pas s'écartez de $\pm 20\%$ du poids moyen du groupe considéré.
- *Identification* : les animaux sont identifiés individuellement par cage, par marquage à l'acide picrique : l'emplacement du marquage, différent pour chaque animal, correspond à un numéro. Un marquage caudal représenté par un trait de couleur au marqueur permet d'identifier le lot.

Hébergement : les animaux sont hébergés à raison de 5 par cage, dans des cages en polycarbonate de 31 cm x 46 cm x 19 cm munies d'un couvercle en inox.

La litière renouvelée régulièrement, est constituée de sciure livrée dépoussiérée et stérilisée aux rayons γ . Elle est fournie par SICSA (94142 Alfortville, France).

Les cages sont placées dans un local d'accès limité, de 5 m x 4.5 m x 3 m, maintenu en légère surpression (10 mm d'eau minimum), sous air régulé en température ($t = 22 \pm 2^\circ\text{C}$) et humidité relative contrôlée (HR = $50 \pm 20\%$) excepté pendant les cycles de lavage et dont le renouvellement en air neuf filtré (sur filtre absolu) se fait à raison de 10 cycles par heure environ.

L'éclairage artificiel assure 12 heures de lumière par jour et 12 heures d'obscurité.

Nourriture : l'aliment complet est fourni sous forme de granulé AO4-10, livré stérilisé aux rayons γ par UAR (91360 Epinay sur Orge, France). Une fiche de contrôle est fournie.

Boisson : l'eau du robinet acidifiée (pH = 2.5) est distribuée en biberons en polypropylène munis d'une tétine en inox.

Un échantillon d'eau est prélevé après toute intervention technique sur la canalisation et au minimum tous les 6 mois et envoyé pour analyse physico-chimique et bactériologique à un organisme de contrôle spécialisé.

5. MODE OPERATOIRE

5.1. Préparation testée

Le produit testé est mis en suspension dans l'eau PPI par agitation manuelle.

Chaque préparation faite **extemporanément** chaque jour en quantité suffisante pour les besoins de l'essai est maintenue sous agitation magnétique pendant toute la durée des traitements. Le mode de préparation est reporté dans le document de travail réservé à cet effet.

La préparation à tester est transférée en salle d'étude selon les modalités définies dans la procédure et mise en place au sein de l'Installation d'Essai.

5.2. Niveaux de dose - Nombre de lots

Les niveaux de dose sont choisis en fonction des résultats obtenus au cours d'un essai préliminaire réalisé au sein de l'Installation d'Essai sur 6 animaux (3 mâles et 3 femelles) recevant une dose de 1000, 500 et 250 mg/kg/jour pendant 7 jours consécutifs et de la détermination de la toxicité aigüe (supérieure à 2000 mg/kg)

Doses choisies :

- dose forte : **1000 mg/kg/jour**
- dose intermédiaire : **200 mg/kg/jour**
- dose faible : **40 mg/kg/jour**

La dose la plus élevée (1000 mg/kg/jour) correspond à la dose maximale pouvant être administrée dans de bonnes conditions (10 ml/kg en suspension). Ce niveau de dose est préconisé dans le cas d'essai limite.

La dose faible (40 mg/kg/jour), correspondant à environ 15 fois la dose journalière administrée à l'homme, ne doit pas produire d'effets nocifs observables. La dose intermédiaire (200 mg/kg/jour) correspond à la moyenne géométrique des deux précédentes (facteur d'incrémentation de 5).

Parallèlement aux lots traités, un lot témoin est constitué. Les animaux de ce lot sont manipulés exactement de la même manière que les animaux des lots traités. Ils reçoivent le véhicule (eau PPI) sous un volume de **10 ml/kg/jour**.

5.3. Administration de la préparation

La préparation à tester est administrée aux animaux quotidiennement, environ à la même heure (entre 9:00 et 11:00 a.m.), sept jours par semaine, sur une période de 28 jours consécutifs au moins. La plage horaire de réalisation des gavages est reportée dans le document de travail réservé à cet effet.

Le volume par kg de poids corporel étant défini, et ce de façon constante pour tous les niveaux de dose (**10 ml/kg**), les volumes de la suspension à tester sont ajustés pour chaque rat, hebdomadairement (à J1, J8, J15 et J22) sur le critère de la dernière pesée.

La préparation est administrée en une fois, par voie orale, à chaque animal, par gavage à l'aide d'une seringue de volume adapté munie d'une canule de taille appropriée.

5.4. Contrôle de concentration

A J1, J15 et J28 avant le gavage et après préparation des solutions à administrer, un prélèvement de chaque préparation à concentration différente est effectué.

Ces prélèvements sont recueillis dans des microtubes en plastique de 1.5 ml jetables, étiquetés, datés et expédiés congelés au Laboratoire d'analyse du donneur d'ordre où les dosages sont réalisés selon la technique appropriée.

Ces prélèvements ont pour but de vérifier la concentration en actif des préparations administrées .

5.5. Examens cliniques

5.5.1. Mortalité

Tous les animaux font l'objet d'un constat de morbidité et de mortalité, 2 fois par jour (matin et soir), hormis les jours chômés (1 seule fois, le matin).

Les animaux moribonds ou présentant des signes de détresse ou de souffrance sévère sont euthanasiés par injection de Pentobarbital® sodique à 6 % (i.p.) puis saignés et autopsiés.

5.5.2. Observation clinique

La période d'observation est de 28 jours minimum.

Un examen clinique général est effectué une fois par jour (au même moment de la journée) avant traitement puis dans l'heure qui suit le gavage.

Un examen clinique approfondi de tous les animaux est effectué :

- avant tout traitement (J1/T0)
- et une fois par semaine au cours de l'essai (J1, J8, J15, J22 et J28 dans l'heure qui suit le gavage).

Les examens sont réalisés hors de la cage sur une aire standard et toujours au même moment de la journée.

Les différents paramètres observés sont les suivants : modification de l'état de la peau, de la fourrure, des yeux, des muqueuses, apparition de sécrétions et d'excrétions et de réactions neurovégétatives (larmoiement, piloérection, variation du diamètre pupillaire, variation du rythme respiratoire...), modifications dans la démarche, la posture, la réaction à la manipulation, présence de mouvements cloniques ou toniques, comportements stéréotypés ou anormaux. La réactivité sensorielle à divers stimuli (auditifs, visuels, proprioceptifs), la force de préhension et l'activité motrice sont appréciées selon des modes opératoires propres au laboratoire d'Essai.

Les observations fonctionnelles ne sont pas faites pour des lots qui manifestent des signes de toxicité à même de perturber les résultats des essais fonctionnels.

Les résultats des observations cliniques sont consignés individuellement dans le document de travail réservé à cet effet.

5.5.3. Poids corporel

Les animaux sont pesés régulièrement : pendant la période d'acclimatation puis à J1, J8, J15, J22 et J28 juste avant administration de la préparation, soit une fois par semaine pendant l'essai.

Le jour de l'autopsie (J29), les animaux sont pesés à jeun.

Les résultats des pesées sont reportés dans le document de travail réservé à cet effet.

5.5.4. Consommation de nourriture

La quantité de nourriture consommée est évaluée par cage sur une période de 48 heures, hebdomadairement par différence de pesée entre la quantité d'aliment fournie et la quantité d'aliment restant dans les mangeoires.

Les résultats sont exprimés en grammes d'aliment consommé/24 heures/100 g de poids corporel.

Toutes les données sont consignées dans le document de travail réservé à cet effet.

5.6. Examens de laboratoire

5.6.1. Hématologie

Au terme de l'essai, tous les animaux sont mis à jeun (J28) le soir (diète hydrique). Le lendemain matin (J29), des échantillons de sang sont prélevés au niveau du sinus rétro-orbitaire de ces animaux sous anesthésie légère (Clorkétam® i.m.).

Les examens hématologiques suivants sont effectués selon les procédures internes à l'Installation d'Essai sur les prélèvements recueillis :

- sur EDTA : hématocrite, teneur en hémoglobine, comptage des érythrocytes, comptage total et différentiel des leucocytes, comptage des plaquettes, constantes érythrocytaires (VGM, TGMHb, CGMhb),,
- sur Citrate : temps de coagulation (temps de Quick).

Toutes les données sont consignées dans le document de travail réservé à cet effet.

5.6.2. Biochimie sérique

Après le recueil de sang pour les examens hématologiques, les animaux sont anesthésiés par injection de Pentobarbital® sodique (i.p.) et des échantillons de sang sont prélevés au niveau de l'aorte abdominale.

Le sang est recueilli sur tubes secs, en vue d'effectuer les dosages biochimiques suivants, selon les procédures internes à l'Installation d'Essai : glucose, créatinine, urée, protéines totales, albumine, cholestérol total, aspartate aminotransférase, alanine aminotransférase, phosphatase alcaline, calcium, sodium, potassium.

Tous les animaux, y compris ceux euthanasiés pendant l'étude, font l'objet de contrôles biochimiques.

Toutes les données sont consignées dans le document de travail réservé à cet effet.

5.7. Anatomopathologie

5.7.1. Autopsie générale

Après recueil du sang pour les examens de biochimie sérique, les animaux sont euthanasiés par saignée au niveau de l'aorte abdominale.

Tous les animaux utilisés dans l'essai (euthanasiés, ou trouvés morts en cours d'essai) font l'objet d'une autopsie générale complète et détaillée qui comporte un examen approfondi des surfaces externes du corps, de tous les orifices et des cavités crânienne, thoracique et abdominale ainsi que de leur contenu.

Le foie, les reins, les surrénales, les testicules, les épididymes, le thymus, la rate, le cerveau et le cœur de tous les animaux (sauf ceux euthanasiés ou trouvés morts au cours de l'essai) sont débarrassés de tout reste de tissu adhérent et leur poids humide est déterminé dès que possible après la dissection. Les organes pairs sont pesés séparément.

Le poids de ces organes est exprimé en valeur absolue et en valeur relative.

La pesée du cerveau est la pesée de référence utilisée dans le calcul du poids relatif des organes.

Chez tous les rats utilisés dans l'essai (euthanasiés, ou trouvés morts en cours d'essai), les tissus ou organes suivants sont prélevés et fixés dans du liquide de Bouin : **tous les organes présentant des lésions macroscopiques importantes, encéphale** (cerveau, cervelet et protubérance annulaire), **moelle épinière, nerf sciatique** (près du muscle), glandes salivaires, **estomac, intestin grêle, gros intestin** (y compris les plaques de Peyer), **foie, rate, moëlle osseuse** (au niveau sternal), **ganglions lymphatiques mésentériques, thymus, cœur, aorte, trachée, poumons, thyroïde/parathyroïdes, surrénales, pancréas, hypophyse, reins, vessie, gonades, épидidymes, organes sexuels accessoires** (utérus, prostate), vagin, glandes mammaires, os (fémur), muscle de la cuisse.

5.7.2. Histopathologie

Après inclusion en blocs de paraffine, toutes les lésions macroscopiques, les organes et tissus prélevés (indiqués en **gras** dans le texte) de tous les animaux du lot témoin et du lot traité à la dose la plus élevée coupés à 4 microns d'épaisseur environ puis colorés à l'hémalun éosine font l'objet d'un examen histologique complet.

Cet examen est pratiqué pour tous les lots exposés à des doses inférieures si des modifications induites par la préparation à tester sont observées chez les animaux du lot traité à la dose la plus élevée.

5.8. Résultats

Les résultats sont indiqués pour chaque animal individuellement et sont récapitulés sous forme de tableaux indiquant par lot :

- le nombre d'animaux à l'essai
- le nombre d'animaux morts au cours de l'essai ou euthanasiés pour des raisons humanitaires
- le moment de la mort ou du sacrifice
- le nombre d'animaux présentant des signes de toxicité
- la description de ces signes, le moment de leur apparition, leur durée et leur gravité
- le nombre d'animaux présentant des lésions
- le type de lésions et le pourcentage d'animaux présentant chaque type de lésion
- l'évolution pondérale
- la consommation en nourriture
- les données, hématologiques, biochimiques et anatomopathologiques.

Les résultats de l'évolution pondérale sont analysés séparément pour chaque sexe par une analyse de variance pour mesures répétées dans le temps prenant en compte les facteurs « temps » et « traitement ».

En présence d'un effet statistiquement significatif, la moyenne du groupe témoin est comparée à chaque moyenne des groupes traités, par le test de Fisher.

Les résultats des examens hématologiques et des examens biochimiques sont analysés séparément paramètre par paramètre.

Après vérification de l'homogénéité des variances entre les groupes (vérification des coefficients de variation), les moyennes sont comparées par une analyse de variance.

En présence d'un effet statistiquement significatif, le groupe témoin est comparé à chacun des groupes traités par le test de Fisher.

Si les conditions d'application du test d'analyse de variance ne sont pas respectées, un test non paramétrique est utilisé. En présence d'un effet statistiquement significatif, chaque groupe traité est comparé au groupe témoin par le test de Mann - Whitney.

Les poids moyens des tissus et des organes prélevés le jour de l'autopsie sont analysés séparément pour chaque sexe selon une démarche identique à la précédente.

Les résultats des observations cliniques quotidiennes, de la consommation alimentaire et des observations macroscopiques d'organes le jour du sacrifice font l'objet d'un commentaire mais ne sont pas analysés statistiquement.

6. RECUEIL DES DONNEES

Toutes les données recueillies au cours de l'essai sont consignées par le technicien en charge de l'étude, sur les documents réservés à cet effet.

Chaque page de ces documents est paraphée et datée par le technicien responsable de l'étude. Toute donnée manquante est justifiée et toute correction est paraphée et datée.

En fin d'étude, les documents de travail, le rapport d'essai ainsi que les spécimens histologiques sont archivés pendant 10 ans maximum dans la salle d'archives de l'Installation d'Essai.

A l'issue de cette période, l'Installation d'Essai définit avec le donneur d'ordre la poursuite de l'archivage, la restitution ou la destruction des données et des spécimens histologiques.

7. ASSURANCE QUALITE

Le Service Qualité vérifie par des audits en cours d'essai que le protocole et les procédures de travail relatives à ce type d'essai sont strictement respectés.

Le Service Qualité réalise régulièrement des audits portant sur l'Installation d'Essai conformément à la procédure correspondante.

Les données expérimentales et le rapport d'essai sont audités conformément à la procédure mise en place au sein de l'Installation d'Essai.

Toute modification au protocole fait l'objet d'un amendement signé par le donneur d'ordre et le Directeur de l'étude.

8. RAPPORT D'ESSAI

Toute anomalie dans le déroulement de l'essai est consignée par écrit et signalée au donneur d'ordre.

Un rapport détaillé, signé par le Directeur de l'étude, le Directeur de l'Installation d'Essai et un membre du Service Qualité est adressé au donneur d'ordre au terme de l'étude.

Ce rapport, conformément aux exigences des BPL, contient notamment :

- le schéma expérimental (avec en particulier la justification du choix du véhicule, des doses, des paramètres biochimiques et hématologiques étudiés et des organes ou tissus prélevés, les détails sur la préparation, son administration),
- les tableaux de résultats, leur commentaire et l'analyse statistique des données chiffrées,
- la discussion et les conclusions du Directeur de l'étude,
- la déclaration du Directeur de l'étude et du membre du Service Qualité.

9. CALENDRIER D'EXECUTION

Début de l'expérimentation : semaine 28

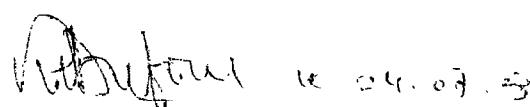
Fin de l'expérimentation : semaine 32

Fin examen histologique : semaine 39

Rapport d'essai (épreuve) : semaine 43

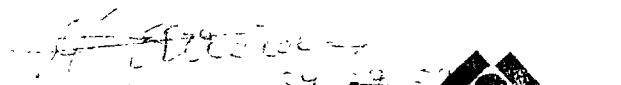
SIGNATURES :

Directeur de l'étude : **Ph. DUFOUR**



Ph. DUFOUR le 04.07.97

Assurance Qualité : **M. DARRICAU**



M. DARRICAU

Donneur d'ordre : **C. LEFRANC**



C. LEFRANC

